THE METABOLISM OF ASCORBIC ACID IN RHEUMATOID ARTHRITIS.

A Thesis presented for the Degree of

Doctor of Philosophy

by

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INTRODUCTION.

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Effect of prolonged ascorbic acid deprivation on collagen formation.

It has been recognised for many years that a failure to form collagen is one of the characteristic features of prolonged ascorbic acid deprivation. As early as 1753 Lind, in his "Treatise of the Scurvy", recorded the observation that one of the symptoms of scurvy was that wounds failed to heal. He noted also that a "peculiar laxness" of the tissues was found at autopsy - a condition now known to be due to the failure in collagen formation. Wolbach and his associates, from histological studies in guinea-pigs which had been rendered scorbutic, concluded that the primary defect in scurvy was a failure of the fibroblasts to form collagen (Wolbach and Howe, 1926; Wolbach, 1933; Boyle, Bessey and Wolbach, 1937). The addition of orange juice to the diet of the experimental animals caused a prompt resumption of collagen formation.

Hunt (1941) observed that collagen does not form in experimental wounds produced in guinea-pigs which have suffered prolonged vitamin C deprivation and that the intercellular material remains immature. Collagen of normally-healing wounds reverts to pre-collagen when scurvy intervenes. Penny and Balfour (1949) observed in healing wounds in scorbutic guinea-pigs that some fibroblastic proliferation occurs but the fibroblasts do not form syncytia and there is very little extra-cellular material. If ascorbic acid is given normal fibroblasts appear and fine reticular fibres and much extracellular material is found. Crandon and Lund (1940), however, found that in a human subject, after 4 months on a vitamin C-free diet, an

experimental wound healed normally even though the plasma, leucocyte and platelet ascorbic acid had fallen to zero. After a longer period of deprivation there was failure to heal in an experimental wound and microscopic examination showed a lack of intercellular material. Parenteral administration of ascorbic acid caused healing to commence and considerable intercellular material appeared in 10 days.

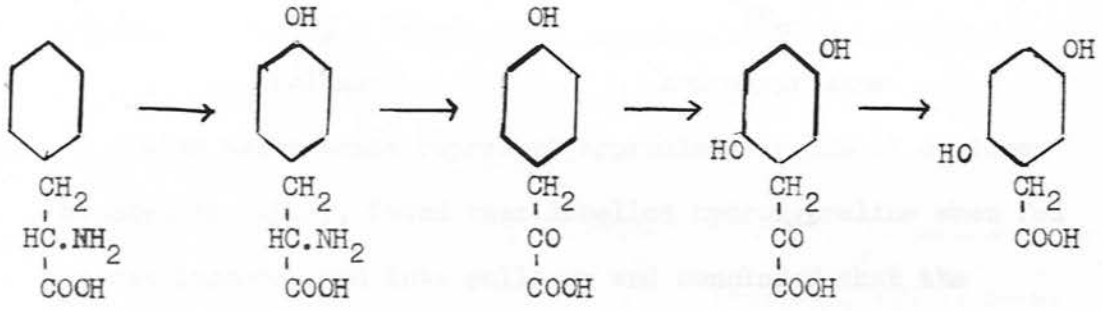
The part played by ascorbic acid in collagen formation is still unknown although Penney and Balfour (1949) observed that an acid mucopolysaccharide was produced in healing wounds in guinea-pigs but that this acid mucopolysaccharide was not produced in scorbutic animals. This mucopolysaccharide is possibly hyaluronic acid which is a component of connective tissue. Elster (1950) investigated the collagen content of the tissues of ascorbic acid depleted guinea-pigs and found no difference in collagen content between these animals and weight controls. He concluded that ascorbic acid is not necessary for maintaining collagen once formed but is possibly necessary for replacement of loss. If this view is correct it could explain the long time necessary for failure of wound-healing to develop in Crandon's human subject. Burns, Burch, and King (1951) fed 1-C^{14}-L-ascorbic acid to guinea-pigs and could detect no radio-activity in chondroitin sulphate or collagen isolated from skin and cartilage. It would appear, therefore, that ascorbic acid does not serve as a precursor or a component of collagen.

A possible explanation of the failure of collagen-formation in the scorbutic state is provided by the effect of ascorbic acid

deficiency on the metabolism of phenylalanine and tyrosine. In the inborn error of metabolism known as alcaptonuria homogentisic acid - 2:5 dihydroxyphenylacetic acid - appears in the urine, although it is not a normal constituent of urine, and the amount of homogentisic acid excreted increases if either phenylalanine or tyrosine is fed to the patient. It would appear therefore that homogentisic acid is an intermediate substance in the metabolism of phenylalanine and tyrosine and that the defect in the alcaptonuric subject is that this metabolite is not oxidised further.

Sealock and Silberstein (1940) observed that scorbutic guinea-pigs excreted p-hydroxyphenyl compounds in the urine and Levine, Gordon and Marples (1941) found p-hydroxyphenyllactic acid and p-hydroxyphenylpyruvic acid in the urine of premature infants fed on cows milk mixtures which were rich in protein but contained no vitamin C. The presence of abnormal amounts of p-hydroxyphenyl compounds has since been noted in the urine of adults with scurvy and also in the urine of patients suffering from pernicious anaemia, rheumatoid arthritis, and steatorrhoea (Rogers and Gardner, 1949; Swendseid, Burton and Bethel, 1943; Boscott and Cooke, 1954). Boscott and Cooke found that patients with steatorrhoea excreted large quantities of p-hydroxyphenylacetic acid in the urine and occasionally p-hydroxyphenyllactic acid was also present. Administration of phenylalanine or tyrosine causes the excretion of increased amounts of these p-hydroxyphenyl compounds while administration of ascorbic acid brings about their disappearance from the urine. Administration of ascorbic acid has no effect on the excretion of homogentisic acid in congenital alcaptonuria.

One pathway of metabolism of phenylalanine and tyrosine which has been suggested is:-

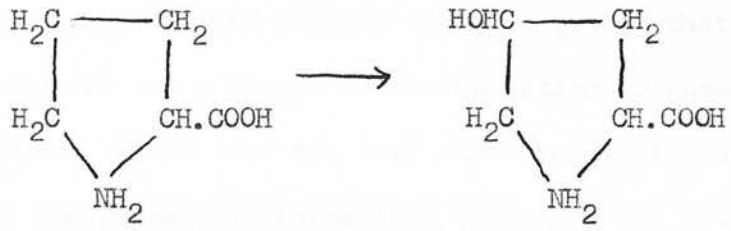


phenylalanine — tyrosine — p-hydroxyphenylpyruvic acid — 2:5 dihydroxyphenylpyruvic acid — 2:5 dihydroxyphenylacetic acid

and thence by opening of the benzine ring and oxidation to acetoacetic acid. Since in ascorbic acid deficiency p-hydroxyphenylpyruvic acid and its derivatives p-hydroxyphenyllactic acid and p-hydroxyphenylacetic acid, are excreted in the urine it would appear probable that ascorbic acid is necessary for the hydroxylation of p-hydroxyphenylpyruvic acid to 2:5 dihydroxyphenylpyruvic acid. There is experimental evidence in support of this view.

Knox and Le-May Knox (1951), using enzyme systems prepared from rat liver have shown that tyrosine may be converted to acetoacetic acid in such systems. p-hydroxyphenylpyruvic acid, 2:5 dihydroxyphenylpyruvic acid and homogentisic acid are also converted to acetoacetic acid. α-oxoglutarate and ascorbic acid are necessary to activate the enzyme system in the conversion of tyrosine to acetoacetic acid; α- oxoglutarate is necessary for deamination of tyrosine and ascorbic acid catalyses the hydroxylation of p-hydroxyphenylpyruvic acid to 2:5 dihydroxyphenylpyruvic acid.

It is possible therefore that ascorbic acid may be necessary for the hydroxylation of proline to hydroxyproline.



proline hydroxyproline

These amino-acids represent approximately 30% of collagen and Stetten (1949), found that labelled hydroxyproline when fed was not incorporated into collagen and concluded that the hydroxyproline of collagen is derived from proline. Gould and Woessner (1957) found that in granulation tissue from regenerating skin in guinea-pigs there was considerable formation of hydroxy-proline. If, however, the animals were deprived of ascorbic acid for seven days there was almost complete cessation of hydroxyproline formation. Administration of ascorbic acid resulted in the formation of relatively large amounts of hydroxyproline within 24 to 48 hours.

Ascorbic acid metabolism in collagen diseases.

The well-established fact of the failure of collagen formation in prolonged ascorbic acid deficiency naturally gives rise to speculation about a possible connection between ascorbic acid deficiency and the so-called "collagen diseases" in which group rheumatoid arthritis is included. From the observations of Crandon and Lund, however, it would appear that failure of collagen-formation as scurvy develops does not occur until even the leucocytes are depleted of ascorbic acid and there is no evidence of such a marked deficiency being a common feature in patients with rheumatoid arthritis.

In 1933 Rinehart and Mettier first suggested that a deficiency of ascorbic acid was a factor in the causation of rheumatic fever and in further papers over the next three years Rinehart and his associates developed this conception (Rinehart and Mettier, 1934; Rinehart, Connor and Mettier, 1934; Rinehart, 1935, 1936). Their conclusions were based on (1) the pathological changes found in guinea-pigs which had been inoculated with streptococcus after being maintained on a diet deficient in vitamin C, (2) a comparison of the joint changes in scurvy and rheumatic fever, and (3) considerations of the seasonal, geographical and social incidence of the disease. These workers suggested that a near-scurvy condition might also play a part in the aetiology of the more chronic condition of rheumatoid arthritis.

These conclusions were not supported by any evidence based on biochemical investigation but in 1936 Rinehart, Greenberg and Baker reported the finding of low values of plasma ascorbic acid in patients suffering from rheumatoid arthritis. The plasma ascorbic acid rose to normal levels after treatment with large doses of ascorbic acid but it was noted that many cases were refractory to such treatment and that there appeared to be an increased requirement of ascorbic acid to maintain normal plasma levels. Similar findings in children with rheumatic fever were reported by Rinehart, Greenberg and Christie (1936). Low values for plasma ascorbic acid, as compared with levels in control groups, and delay in reaching normal levels on treatment with large doses of ascorbic acid were reported in rheumatic fever patients (Rinehart, Greenberg, Olney and Choy, 1938), in patients with rheumatoid arthritis (Rinehart,

et al, 1938) and in patients with rheumatoid spondylitis, (Rinehart, 1939). In a study of 55 patients with arthritis Rinehart and his co-workers observed that it was in arthritis of the rheumatoid type that plasma ascorbic acid levels were low (Rinehart, Greenberg, Baker, Mettier, Bruckman and Choy, 1938). Low levels of plasma ascorbic acid in rheumatoid arthritis have been reported also by Hall, Darling and Taylor (1939), Secher (1940), Jacques (1940), and Freyburg (1942).

As early as 1936 Abassy, Gray Hill and Harris had noted a low urinary excretion of ascorbic acid, as compared with normal controls, in children with rheumatic fever, and similar findings were reported by Abassy and Harris (1937) and Hare and Williams (1938). Keith and Hickmans (1938), however, found that in children with rheumatic fever the 24 hour excretion of ascorbic acid was higher than in normal controls but, since salicylate therapy was not suspended, the higher ascorbic acid excretion was almost certainly due to the effect which this drug has on urinary excretion of ascorbic acid (Spitzer and Shapiro, 1948).

Perry (1935), Schultz, Sendroy and Swift (1935) and Sendroy and Schultz (1936) measured the urinary excretion of ascorbic acid in patients with rheumatic fever before and during a period on a fixed test dose of ascorbic acid and concluded that there was no evidence that a deficiency of vitamin C played any part in the aetiology of the disease, although the experimental results of both Perry and Sendroy and his co-workers indicated an increased requirement for the vitamin in rheumatic fever. Schultz (1936) repeated the experiments of Rinehart and his associates on guinea-

pigs rendered scorbutic and infected with streptococcus and expressed the opinion that the bone lesions produced only superficially resembled those found in rheumatic fever. Bayles, Richardson and Hall (1943) investigated the nutritional background of 31 patients with rheumatoid arthritis and could find no evidence that a previous dietary deficiency was responsible for the onset of the disease.

Schroeder (1935), Harde, Rothstein and Ratish (1935), Bullowa, Rothstein, Ratish and Harde (1936) and other workers have shown that a deficiency state with regard to vitamin C exists in a variety of infectious conditions. Doubtless these reports, along with the lack of evidence of any amelioration produced by treatment with the vitamin in rheumatic conditions, was the chief reason for the investigation of the metabolism of ascorbic acid in rheumatoid arthritis not being pursued further. The view was fairly generally accepted that the low plasma levels and low urinary excretion of the vitamin in rheumatic fever was a phenomenon common to most infective conditions. However this may be true in the case of rheumatic fever there is no definite evidence that rheumatoid arthritis is due to an infection.

Ascorbic acid and adreno-cortical function.

Szent-Gyorgyi first showed in 1928 that there is a high concentration of ascorbic acid in the adrenal gland. Previously, in 1926, Randoin and Michaux had reported that the cholesterol content of the gland was reduced in scurvy and Harris and Ray (1933) observed that the ascorbic acid content of the adrenal was reduced in scurvy. Giroud et al (1940) suggested that

ascorbic acid was necessary for the formation of adreno-cortical hormones from cholesterol. Long and his co-workers (Sayers, Sayers, Lewis and Long, 1944; Sayers, Sayers, Liang and Long, 1945 and 1946) showed that ACTH depleted the adrenal cortex of rats of both ascorbic acid and cholesterol. Haemorrhage had the same effect but was without effect if the pituitory had previously been removed. These workers suggested that haemorrhage and other forms of stress stimulated the pituitary and that the consequent increased secretion of ACTH stimulated the adrenal cortex to produce cortico-steroids from cholesterol using ascorbic acid in the process.

Some experimental evidence has been advanced in support of the theory that ascorbic acid is necessary for the production of the hormones of the adrenal cortex. Schaffenburg, Masson and Corcoran (1950) reported that cortisone inhibited many of the manifestations of scurvy in guinea-pigs maintained on a vitamin C-free diet and similar findings were reported by Herrick et al (1952). Stefanini and Rosenthal (1950) and Holley and McLester (1951) reported cases in which ACTH given to human patients over a long period had caused the development of symptoms similar to those of scurvy, although the evidence presented for the presence of scurvy was not very convincing. The inference would be that continued production of cortico-steroids under the stimulation of ACTH had exhausted the body reserves of ascorbic acid. The increased requirement for ascorbic acid observed in infectious conditions and following burns (Lund et al, 1947) might be taken as evidence in favour of such a theory. Itis known that

conditions of stress increase the production of adrenocorticotrophic hormone (ACTH) by the pituitary and hence the production of corticosteroids. If ascorbic acid is necessary for the synthesis of these hormones this increased production of hormones would give rise to an increased requirement for ascorbic acid. Schmidt and Staudinger (1954) have reported that ascorbic acid, and also dehydroascorbic acid, activates the formation of corticosteroids in systems prepared from pigs adrenals.

On the other hand Upton and Coon (1951) and Clayton and Prunty (1951) did not find that cortisone or ACTH had any effect on the course of experimental scurvy in guinea-pigs. In fact, Clayton and Prunty found an increased excretion of 17-ketosteroids in scorbutic guinea-pigs which would suggest an increased production of adreno-cortical steroids in spite of the ascorbic acid depletion. Hyman, Ragan and Turner (1950) reported that both cortisone and ACTH prolonged life and reduced the haemorrhagic manifestations of scurvy in guinea-pigs. There has been no reliable evidence that patients suffering from well-marked symptoms of scurvy show symptoms of adreno-cortical insufficiency such as are manifested in Addison's disease, and Treager et al (1950) reported that patients with marked clinical scurvy gave a normal response to the ACTH test (as evidenced by eosinophil count and serum electrolytes). Finally, there is the observation of Wolbach and Maddock (1952) that cortisone has no effect on collagen-formation in scorbutic guinea-pigs.

When beneficial results were obtained in rheumatoid arthritis by treatment with cortisone it was natural to infer a connection

between rheumatoid arthritis and adreno-cortical activity and, if the production of the hormones of the adrenal cortex depended on a supply of ascorbic acid, this would imply a connection between ascorbic acid metabolism and rheumatoid arthritis. However, in the light of the conflicting evidence quoted a connection between ascorbic acid and adreno-cortical steroid production is far from being proved. Another possible connection between ascorbic acid and rheumatoid arthritis was introduced when Lewin and Wassen, in 1949, claimed that simultaneous injections of ascorbic acid and deoxycorticosterone acetate (DOCA) produced clinical improvement in cases of rheumatoid arthritis. Similar reports of beneficial results from the simultaneous injections of ascorbic acid and DOCA were made by Le Vay and Loxton (1950) and Littman, Stockdale and Williamson (1951) and it was suggested that the effects of this combination were due to the conversion in the body of DOCA to an unspecified adreno-cortical hormone by ascorbic acid, (Hallberg, 1950). If such a conversion does occur it may be another instance of ascorbic acid being necessary for hydroxylation, in this case the introduction of a hydroxyl group at carbon atom 17. However, the investigations of Spies et al (1949), Bywaters, Dixon and Wild (1950), Copeman et al (1950) and others failed to substantiate the therapeutic effect claimed for the combination of ascorbic acid and DOCA and it was found that it produced none of the biochemical effects of cortisone.

There would appear therefore to be little evidence that a deficiency in respect to ascorbic acid is a causative factor in rheumatoid arthritis, that treatment with large doses of the vitamin

has any therapeutic effect in the condition - although Massell et al (1950) claimed to have brought about clinical improvement by dosage of 4 g. ascorbic acid per day - or that the nutritional state with regard to the vitamin affects rheumatoid arthritis through production of adreno-cortical steroids. Nevertheless there were, between 1936 and 1942, repeated reports from various sources of low levels of plasma ascorbic acid, low urinary excretion and apparent increased requirement for the vitamin. A further investigation, using the newer techniques not available to the earlier workers, would appear to be warranted. Such an investigation should have as its objects firstly, to confirm or otherwise the findings of the earlier workers, and secondly, to bring to light any further differences that may exist between rheumatoid arthritis patients and non-rheumatoid subjects with respect to the metabolism of ascorbic acid. If such differences as are shown to exist point to an actual difference in the metabolic pathway of the vitamin in rheumatoid arthritis then an explanation of the nature of this difference in metabolism should be sought.

Criteria of ascorbic acid deficiency.

One method of assessing the state of nutrition with respect to ascorbic acid which has been extensively used is the ascorbic acid saturation test. A fixed dose of the vitamin, usually 500 mg. or else 10 mg. per kilogram body weight, is given daily until "saturation", as shown by the excretion in a 24 hour period of an arbitrarily chosen amount of ascorbic acid, is reached.

The concept of "saturation" as applied to ascorbic acid has

been criticised (Bicknell and Prescott, 1953) but, while it is true that a plasma ascorbic acid level such as it is necessary to attain before a sharp rise in urinary excretion occurs has never been shown to be physiologically necessary for the maintenance of health, yet the number of days required to reach such a level on a fixed daily dose of ascorbic acid does provide a rough indication of the state of nutrition of the subject with respect to the vitamin. The criticism has also been made that the conditions under which these tests are carried out vary so much that no conclusions can be taken from the data obtained but this objection is not valid if, in a single investigation, all the tests are carried out under the same conditions. A series of such tests on rheumatoid arthritis patients and non-rheumatoid controls might provide evidence as to whether there does indeed exist a deficiency of, or increased requirement for, ascorbic acid in rheumatoid arthritis.

Many of the earlier workers cited a lower level of ascorbic acid in the plasma of rheumatoid arthritis patients as evidence of a deficiency of the vitamin. Confirmation of this must be sought but the methods used by these workers estimated ascorbic acid only, and, as has been shown by Stewart, Horn and Robson (1953), dehydroascorbic acid is also present normally in blood plasma and account must therefore be taken of the amount of both substances present in the plasma of rheumatoid arthritis patients and normal controls. The level in the blood plasma however, may not be the best index of the state of ascorbic acid nutrition or of body storage of the vitamin and some consideration must be given to this aspect of the problem.

Roe, Kuether and Zimler, in 1947, observed that the level of ascorbic acid in the plasma was lower than that of the erythrocytes when the level of ascorbic acid in both plasma and leucocytes was low but that these levels tended to reachequality as the ascorbic acid concentration in the blood increased, and Damron, Monier and Roe (1952) found that as the total ascorbic acid (i.e. ascorbic acid plus dehydroascorbic acid plus dioxogulonic acid) in all the tissues of guinea-pigs decreased in ascorbic acid deprivation it was in the amount present as ascorbic acid that the greatest decrease occurred.

It has been shown (Lloyd, 1951; Golden and Sargent, 1952; Lloyd and Parry, 1954; Iggo, Thomson and Stewart, 1958) that the cell wall is readily permeable to dehydroascorbic acid but that ascorbic acid passes through the membrane only slowly. Stewart, Robson and Horn found (unpublished data) that dehydro-ascorbic acid was not formed in the plasma and it would appear from the experiments of Heinemann(1941) that the presence of leucocytes causes the conversion of ascorbic acid to dehydroascorbic acid which is then capable of passing into the erythrocytes where it is reduced again. It would appear therefore that the analysis of leucocytes or erythrocytes can provide no clear index of body store of the vitamin but since Crandon, Lund and Dill (1940) found that the appearance of scorbutic symptoms in prolonged ascorbic acid deprivation was preceded by the virtual disappearance of ascorbic acid from the leucocytes the level of ascorbic acid in the leucocytes has generally been taken as providing a rough indication of the body storage.

Iggo, Thomson and Stewart (1958) observed that the erythrocytes of rheumatoid arthritis patients reduced dehydroascorbic acid to ascorbic acid at a more rapid rate than did those of non-rheumatoid controls. Since dehydroascorbic acid can pass into the erythrocytes more easily than ascorbic acid can pass out of these cells it would be expected that this more rapid reduction of dehydroascorbic acid would lead to a depletion of the dehydro-ascorbic acid content of the plasma, other factors being equal. The investigation must include, therefore, the estimation of ascorbic acid and dehydroascorbic acid in plasma and erythrocytes, and of total ascorbic acid in leucocytes, of rheumatoid arthritis patients and normal controls.

Some of the earlier workers found evidence of ascorbic acid deficiency in low urinary excretion but there was no uniformity in the intake of the vitamin during their experiments. A comparison of the excretion of ascorbic acid in the 24 hour period after a standard test dose of the vitamin in rheumatoid arthritis patients and controls would appear to be required and the carrying out of such tests on "unsaturated" subjects and on subjects who had already been brought to "saturation" level might yield useful information.

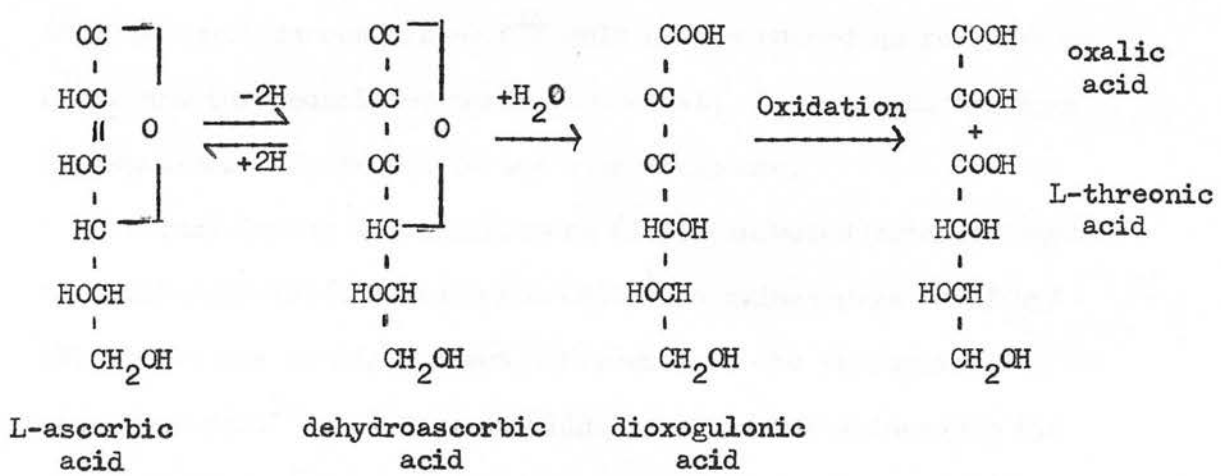
Saturation tests should provide a rough measure of the tissue content, or body "reserve" of ascorbic acid, The plasma levels of ascorbic acid will reflect the recent intake of the vitamin in relation to body requirement while the plasma-erythrocyte ratio should give an indication as to the turnover. The amount of dehydroascorbic acid present, in relation to the "total" ascorbic

acid, in the plasma should indicate any difference in the rate of oxidation of the vitamin. If a difference in the metabolism of ascorbic acid does in fact exist in rheumatoid arthritis such a combination of tests should give some evidence of its existence and perhaps, of its nature.

The metabolic pathway of ascorbic acid.

In considering the question as to a possible difference in the metabolism of ascorbic acid between rheumatoid arthritis patients and the normal physiological condition it is necessary to review briefly what is at present known about the pathway of metabolism of ascorbic acid in the animal body.

It has been known for some time that oxalic acid is one of the products of both the *in vitro* and *in vivo* oxidation of ascorbic acid (Herbert, Hirst, Percival, Reynolds and Smith, 1935; Jurist and Christiansen, 1939; Rosenfeld, 1943). *In vitro* ascorbic acid is reversibly oxidised to dehydroascorbic acid and thence, by rupture of the lactone ring, to dioxogulonic acid. Dioxogulonic acid may then be further oxidised to oxalic acid and L-threonic acid.



In 1951 Burns, Burch and King using L-ascorbic -1-C^{14} acid showed that in rats part of the oxalic acid excreted was derived from injected ascorbic acid and Hellman and Burns (1955) have demonstrated that in man also part of the excreted oxalic acid is derived from ascorbic acid.

There is as yet no clear evidence that the pathway ascorbic acid - dehydroascorbic acid - dioxogulonic acid - oxalic acid is the main pathway in vivo. Damron, Monier and Roe (1952) as a result of their experiments on guinea-pigs gave as their opinion that the reaction ascorbic acid - dehydroascorbic acid - dioxogulonic acid was a "pathway of decomposition" rather than the normal metabolism in animal tissues. Curtin and King (1955) injected L-ascorbic-1-C^{14} acid and similarly labelled dehydroascorbic acid and dioxogulonic acid into guinea-pigs and found the percentage conversion to respiratory $C^{14}O_2$ in 24 hours to be 19%, 29% and 47% respectively. The corresponding conversion to urinary oxalate was 2% with ascorbic acid, 9% with dehydroascorbic acid and 12% with dioxogulonic acid. When they injected oxalate containing C^{14} only 0.4% appeared as respiratory $C^{14}O_2$ and they concluded that, in the rat, there was no evidence of conversion of oxalate to any other compound.

Burns, Dayton and Schulenberg (1956) injected both carboxyl- and uniformly-labelled ascorbic acid into guinea-pigs and found that there was no significant difference in the proportion converted to $C^{14}O_2$. They concluded that in the guinea-pig the main route of metabolism involves the oxidation of the entire carbon chain to carbon dioxide, smaller amounts being excreted

as ascorbic acid, dioxogulonic acid, and oxalic acid. It appears possible, therefore, that in the rat and the guinea-pig the main pattern of metabolism is the oxidation of ascorbic acid via dehydroascorbic acid and dioxogulonic acid to carbon dioxide. Their experiments however provided no evidence as to whether oxalic acid was formed, with L-threonic acid, as intermediate products in the oxidation to carbon dioxide or whether it was the product of an alternative pathway of metabolism and was excreted unchanged. If ascorbic acid is converted to oxalic acid - via dehydroascorbic acid and dioxogulonic acid - and the oxalic acid is immediately oxidised further in some tissue enzyme system then the oxalic acid which reaches the plasma - and thence the excretory mechanism - may be an excess which escapes from the system. In this case any oxalic acid injected into the blood stream may easily be excreted unchanged and the fact that it is so excreted is no proof that oxalic acid formed in the tissues from ascorbic acid is excreted unchanged.

Unfortunately there is less data available regarding the metabolic pathway in man. Hellman and Burns (1955) injected L-ascorbic-1-C^{14} acid intravenously in two human subjects and found that no more than 10% appeared as respiratory $C^{14}O_2$ while approximately 70% was excreted in the urine - approximately 15% of this was ascorbic acid, 15% was dioxogulonic acid and 55% was oxalic acid. These results are surprising in view of the findings of Lambden and Chrystowski that the intake of ascorbic acid had to be raised to over 4 g. per day before any significant increase occurred in the urinary excretion of oxalate. There is no

evidence as yet as to whether oxalic acid is converted in the human body to any other substance or whether all the oxalic acid formed is excreted as such. Archer and his co-workers (1958) gave oxalic acid orally, as sodium oxalate, to two human subjects and calculated the increase in urinary oxalate to represent only 1.9% and 1.2% respectively of the dose given. Since, however, they had no means of determining the levels of oxalate in the blood there is no evidence as to how much of the oxalate which was given was actually absorbed into the blood. It would be expected that most of such a dose, given orally, would form insoluble calcium and magnesium oxalates in the intestine and would therefore not be absorbed. It is known that eating rhubarb leaves or spinach, which contain appreciable amounts of soluble oxalate, tends to lower the body supply of calcium by binding the calcium in the diet as insoluble oxalate.

Whatever the true facts are as to the metabolic pathway of ascorbic acid in man it would seem probable that if a difference in the metabolism of the vitamin does exist in rheumatoid arthritis such a difference may be reflected in some change in the rate of excretion of oxalic acid in the urine. Horn (1956) measured the urinary excretion of oxalate in rheumatoid arthritis patients and control subjects following very large oral doses of ascorbic acid and found that the excretion of oxalate was significantly lower in the rheumatoid than in the control group. Previous workers have reported lower urinary excretion of ascorbic acid in rheumatoid arthritis and the fact that excretion of oxalic acid is also lower in this condition is somewhat surprising, although it could

be explained by a failure in intestinal absorption of ascorbic acid in rheumatoid arthritis. To investigate this possibility and obtain further information about the excretion of oxalate in patients with rheumatoid arthritis it is required that Horn's experiments should be repeated but that the large doses of ascorbic acid should be given by the intravenous route. Another point requiring investigation is whether the state of nutrition of the rheumatoid arthritis patient with respect to ascorbic acid has any effect on the excretion of oxalic acid. In other words, if rheumatoid arthritis patients are brought to "saturation" level with large doses of ascorbic acid do they still excrete less oxalic acid than non-rheumatoid controls.

MATERIAL AND METHODS.

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(1) Subjects.

All the rheumatoid arthritis patients who were subjects for the experiments were patients who were in bed in the Rheumatoid Arthritis Unit wards in the Northern General Hospital. Most of the control subjects were patients in bed in the Neurological Unit at the same hospital who had been in hospital for several weeks and had received no vitamin supplement since admission. The exceptions were the control subjects in measurement of urinary excretion of ascorbic acid after 'saturation' with the vitamin and the control subjects in the experiments where cortisone and salicylate were given; members of the hospital staff were taken as controls for these experiments.

(2) Ascorbic acid content of plasma.

(a) "Total" ascorbic acid.

The "total" ascorbic acid content of the plasma was estimated using the method of Roe and Kuether (1943). In this method a trichloracetic acid extract of the plasma is shaken with an activated charcoal (Norit) to oxidise ascorbic acid to dehydroascorbic acid. The solution is then incubated with a sulphuric acid solution of 2:4 dinitrophenylhydrazine and the resulting coloured compound measured spectrophotometrically. It is probable that the 2:4 dinitrophenylhydrazine couples with dioxogulonic acid and under the experimental conditions there is rapid conversion of dehydroascorbic acid to dioxogulonic acid. The method therefore measures ascorbic acid plus dehydroascorbic acid plus dioxogulonic acid and it is the sum of these substances

that will be referred to as "total" ascorbic acid.

Reagents. 1. 6 g. trichloracetic acid in 100 ml. water.

2. Acid-washed Norit - 200 g. Norit were boiled with 1 litre 10% hydrochloric acid and then filtered by suction. The Norit was transferred to a beaker, 1 litre distilled water was added and after thorough stirring the mixture was filtered. The washing with water was repeated till the filtrate contained no ferric iron. The Norit was then dried at 110° to 120° C.

3. 2:4 dinitrophenylhydrazine solution - 2 g. dissolved in 100 ml. approximately 9 N sulphuric acid.

4. 10 g. thiourea dissolved in 100 ml. ethanol - water (1:1)

5. 85% sulphuric acid - nine parts sulphuric acid added to one part water.

Procedure. One part of plasma was added, slowly and with shaking, to three parts trichloracetic acid, mixed, and allowed to stand for 5 minutes. After centrifugation the supernatant fluid was pipetted off, shaken vigorously for 4 minutes with Norit (approximately 0.1 g. per ml.), and filtered.

4 ml. filtrate was placed in each of two test-tubes and one drop of thiourea solution was added to each tube. 1 ml. of dinitrophenylhydrazine reagent was added to one of the tubes which was then incubated at 37°C. for 3 hours. The second tube was kept as a blank and 1 ml. of the dinitrophenylhydrazine reagent was added to this tube at the end of the three hour period. Both

Table A.

Roe and Kuether method for "total" ascorbic acid.

(1) Recovery of added ascorbic acid in plasma.

	Ascorbic acid added mg./100 ml.	Ascorbic acid found mg./100 ml.	Recovery %
1.	Nil	Nil	-
2.	Nil	Nil	-
3.	Nil	Nil	-
4.	0.80	0.80	100.0
5.	0.80	0.81	101.3
6.	0.80	0.82	102.5
7.	0.80	0.78	97.5
8.	0.80	0.78	97.5
9.	0.80	0.83	103.8

Mean recovery 100.2% S.D. 0.02 mg./100 ml.

(2) Results of replicate determinations.

	Serum X Ascorbic acid mg./100 ml.		Serum Y Ascorbic acid mg./100 ml.
1.	2.29	1.	1.35
2.	2.35	2.	1.39
3.	2.45	3.	1.42
4.	2.42	4.	1.37
5.	2.36	5.	1.30
6.	2.33	6.	1.38
7.	2.36	7.	1.32
8.	2.42	8.	1.44
		9.	1.44
		10.	1.28
Mean	2.37	Mean	1.37

S.D. 0.05 mg./100 ml.

tubes were then immediately placed in an ice and water mixture and 5 ml. of 85% sulphuric acid were added, drop by drop, to the contents of each tube. The tubes were allowed to stand at room temperature for 30 minutes and then the optical density of the test solution was read on the Unicam Spectrophotometer S.P. 350 at 505 mμ, the instrument being set to zero with the blank solution.

With each batch of estimations a series of standard solutions containing 0.25, 0.50, 1.00, 1.50 and 2.00 mg. ascorbic acid per 100 ml. were prepared. A one in four dilution of each standard solution with 6% trichloracetic acid was made and treated in the same way as the plasma filtrates. From the optical densities obtained from these standards a graph was made from which the concentration of "total" ascorbic acid in the plasma could be read.

In a recovery experiment the equivalent of 0.8 mg. ascorbic acid per 100 ml. was added to a mixed specimen of old plasma which appeared to contain no ascorbic acid when tested by the Roe and Kuether procedure. Six determinations were made on the protein-free filtrate and the mean recovery was 100.4% with a standard deviation of 0.02 mg. per 100 ml. Estimations of ascorbic acid were carried out on eight separate samples from one specimen of plasma and on ten separate samples from a second specimen of plasma and the standard deviation from the mean in these eighteen determinations was 0.05 mg. per 100 ml. The details of these experiments are set out in Table A.

(b) Ascorbic acid content.

The ascorbic acid content of plasma was determined by the

method described by Stewart, Horn and Robson (1953) using 2:6 dichlorophenolindophenol. A measured amount of the dichlorophenolindophenol solution is added to an aliquot of a metaphosphoric acid extract of the plasma and the optical density of the unreduced dye is measured in the photo-electric spectrophotometer.

Reagents. 1. 2:6 dichlorophenolindophenol solution - one tablet of B.D.H. dichlorophenolindophenol (stated to contain the equivalent of 1 mg. ascorbic acid) dissolved in 100 ml. water.

2. 3% (w/v) metaphosphoric acid, freshly prepared each day.

Procedure. Two volumes of plasma were added, slowly with shaking to three volumes of 3% metaphosphoric acid solution. After mixing and allowing to stand for a few minutes the mixture was centrifuged and the supernatant fluid filtered.

Into a 10 m.m. cuvette was pipetted 2 ml. filtrate and 1 ml. dichlorophenolindophenol solution. After exactly 30 seconds the optical density was read on the Unicam Spectrophotometer S.P. 350 at 520 mμ. The instrument was set to zero with a blank prepared by adding 1 ml. of dye solution to 2 ml. of plasma filtrate and decolorizing with excess of solid ascorbic acid. A calibration curve was prepared for each batch of estimations from standard solutions of ascorbic acid in 1.8% (w/v) metaphosphoric acid.

In a recovery experiment the equivalent of 0.80 mg. per 100 ml. ascorbic acid was added to old serum. The serum, before addition of ascorbic acid, was shown to contain no ascorbic acid by the

Table B.

Stewart, Horn and Robson method for ascorbic acid.

Recovery of added ascorbic acid in plasma

	Ascorbic acid added ≡ mg/100 ml.	Ascorbic acid found mg./100 ml.	Recovery %
1 (a)	Nil	Nil	-
(b)	Nil	Nil	-
2 (a)	Nil	Nil	-
(b)	Nil	Nil	-
3 (a)	0.80	0.74	92.5
(b)		0.76	95.2
4 (a)	0.80	0.76	95.2
(b)		0.75	93.8
5 (a)	0.80	0.74	92.5
(b)		0.75	93.8

Mean recovery 93.8 % S.D. 0.01 mg./100 ml.

Stewart, Horn and Robson procedure. The mean recovery from duplicate determinations on each of the filtrates from three separate samples of serum was 93.8% and the standard deviation from the mean was 0.01 mg. per 100 ml. The detailed results are given in Table B.

Since this part of the work was completed a recent paper by Iggo, Owen and Stewart (1956) has shown that there is acid destruction of 2:6 dichlorophenolindophenol at the pH used in this method and, since the destruction does not appear to proceed at the same rate in the plasma filtrate as in the standard solution, they point out that this will lead to slightly low results for ascorbic acid. The error found in the recovery experiment is small and the results obtained varied from 0.04 to 0.06 mg. per 100 ml. from the true value; since the results for both rheumatoid arthritis patients and control subjects were obtained by the same method they are comparable.

Owen and Iggo (1955) used p-chloromercuribenzoic acid to remove substances - chiefly thiols and thiosulphates - which interfere in the estimation of ascorbic acid when 2:6 dichlorophenolindophenol is used. They found that when metaphosphoric extracts of human plasma were used the amount of interfering substance removed by p-chloromercuribenzoic acid was negligible and, therefore, it appears reasonable to assume that the substance estimated by this method is almost entirely ascorbic acid.

(3) Ascorbic acid content of erythrocytes.

The "total" ascorbic acid content of the erythrocytes was

obtained by the Roe and Kuether (1943) method.

The ascorbic acid content was determined by reduction of 2:6 dichlorophenolindophenol after saturation of the cells with carbon monoxide. When blood erythrocytes are laked the release of haemoglobin into a medium containing copper ions results in the rapid and complete oxidation of all ascorbic acid present (Lemberg, Legge and Lockwood, 1939). Fujita, Ebihara and Numata (1939) suggested that blood should be saturated with carbon monoxide before estimation of ascorbic acid and a method using this technique was developed by Butler and Cushman (1940). Thomson (1955) adapted the method of Butler and Cushman for the estimation of ascorbic acid in erythrocytes and demonstrated that accurate estimation of ascorbic acid in erythrocytes could be obtained by this method.

(4) Ascorbic acid content of urine.

'Total' ascorbic acid was estimated by the Roe and Kuether method. For the estimation of ascorbic acid the method used was that described by Mapson (1953) as modified by Owen (Personal communication). The method is based on the fact that ascorbic acid condenses with formaldehyde to give a product which does not reduce 2:6 dichlorophenolindophenol; the optimum pH for this condensation is 3.5 and the rate of condensation diminishes rapidly with decreasing pH till at pH 0.6 it is very slow, while interfering reducing substances - sulphydril derivatives, sulphides and thiosulphates - condense rapidly with formaldehyde at pH 0.6.

The 24 hour urine was diluted to 2000 ml., if the volume was less than this, and an aliquot was adjusted to pH 0.6 with 50% (v/v)

sulphuric acid. To this was added $\frac{1}{4}$ of its volume of 40% formaldehyde solution adjusted to pH 0.6 (sample A). Another aliquot of urine was adjusted to pH 3.5 with 30% (w/v) sodium citrate solution and $\frac{1}{4}$ of its volume of 40% formaldehyde adjusted to pH 3.5 added (sample B).

The reducing power of sample A was determined 15 minutes and again at 20 minutes after addition of formaldehyde by adding 2 ml. of sample to 5 ml. of phosphate-citrate buffer, pH 3.5, 1 ml. 30% sodium citrate and 2 ml. dichlorophenolindophenol solution and reading in the photo-electric colorimeter after 30 seconds. The reducing power of sample B was determined in the same way at 15 minutes and 20 minutes after addition of formaldehyde, except that in this case 1 ml. water was substituted for 1 ml. 30% sodium citrate. The readings obtained were plotted on graph paper and by exterpolation the readings of A and B at zero time were obtained. From these readings, corrected for dilution, the reducing power of A and B in terms of ascorbic acid were obtained and the ascorbic acid concentration of the urine was given by A-B. A standard curve, obtained from freshly prepared solutions of ascorbic acid, was made with each batch of estimations.

When a five-day old specimen of urine was examined by this procedure there was no difference in reducing power between the portion treated with formaldehyde at pH 0.6 and that treated at pH 3.5. The equivalent of 1.0 mg. ascorbic acid per 100 ml. was added to a portion of the urine and determinations on three separate samples gave results of 1.03, 0.95 and 0.94 mg. per 100 ml. However the method is time-consuming and, owing to the careful time-control

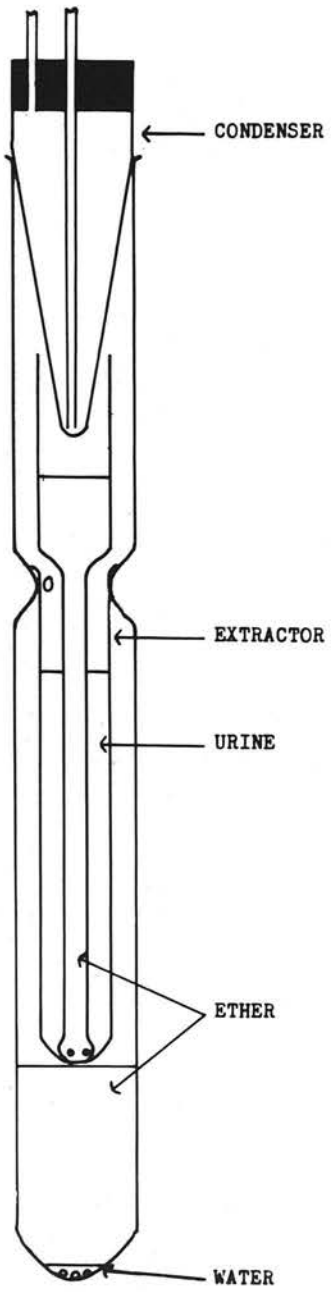
required, there were practical difficulties when a number of specimens had to be estimated at the same time. A further disadvantage was the fact that, when a number of estimations were being carried out simultaneously, there was a tendency for a white precipitate to form in the treated aliquots in spite of the volume of the 24-hour urine being diluted to 2000 ml. Accordingly in most of the experiments 'total' ascorbic only, by the Roe and Kuether procedure, was estimated.

In the estimation of dehydroascorbic acid in urine the dehydroascorbic acid was reduced to ascorbic acid by passing H_2S through the urine, which had previously been adjusted to pH 3.5 with sodium citrate solution, for 30 minutes. The H_2S was then removed from the urine by bubbling nitrogen through it for one hour. Ascorbic acid was then estimated by the modified Mapson procedure and the dehydroascorbic acid content obtained by difference.

(5) Oxalate content of urine.

In the estimation of urinary oxalate the method described by Powers and Levatin (1944) was used. These authors concluded that methods of estimating oxalate in urine by direct precipitation as calcium oxalate were unsatisfactory owing to interference by magnesium, sulphate and phosphate ions. In the method of Powers and Levatin the urine, after heating with hydrochloric acid to hydrolyse any oxaluric acid which may be present to oxalic acid, is extracted with ether in a continuous extraction apparatus. After removal of the ether the oxalic acid is precipitated as calcium oxalate, excess of standard potassium permanganate is

FIGURE 1.



EXTRACTION
APPARATUS

added and the excess permanganate is titrated iodometrically.

Apparatus.

A diagram of the apparatus is shown in Fig. 1. The outer tube is 300 m.m. in length and 25 m.m. in diameter; the inner tube is 120 m.m. in length and 15 m.m. in diameter and is supported inside the outer tube by notches pushed into the glass 115 m.m. from the top of the tube. The funnel-shaped tube has an over-all length of 180 m.m. and the stem end is sealed and has four holes punched through the sealed end with a hot wire. When in position the extractor and the funnel-shaped tube are suspended from the top of the outer tube by lengths of cotton thread to assist in withdrawal at the end of the extraction period. A cold-finger condenser is placed in the top of the outer tube and the cotton thread serves to separate the condenser slightly from the rim of the tube, thus providing a vent.

Reagents.

(1) Concentrated hydrochloric acid.

(2) Di-ethyl ether.

(3) 2% (v/v) acetic acid in water.

(4) Ethanol.

(5) Acid-ethanol solution - 60 ml. ethanol, 10 ml. 2% acetic acid and 20 ml. water.

(6) 10 g. calcium chloride in 100 ml. water.

(7) 20% (v/v) sulphuric acid.

(8) 1 g. manganese sulphate in 100 ml. water.

(9) 10 g. potassium iodide in 100 ml. water.

(10) 0.01 N potassium permanganate solution.

(11) 0.01 N sodium thiosulphate solution.

(12) 1% starch suspension.

Procedure.

The 24 hour urine specimen was thoroughly mixed and 25 ml. were pipetted into a boiling tube. 1 ml. concentrated hydrochloric acid was added, the contents mixed by gentle shaking, and the tube was heated in a boiling water-bath for 30 minutes in order to hydrolyse any oxaluric acid present to oxalic acid. The tube was then cooled and the urine filtered.

In the outer tube of the extraction apparatus a small piece of capillary glass tubing was placed (to prevent "bumping") and 2 ml. water and 25 ml. ether were added. 10 ml. of the filtered, hydrolysed urine were measured into the inner tube and extraction was carried out on an electric hot-plate for 6 hours, the rate of heating being such that the ether dripped at the rate of at least 100 drops per minute.

The apparatus was then disconnected and the inner tube removed after washing down with 2 ml. ethanol. 1 ml. of 2% acetic acid was added to the contents of the outer tube and the ether removed by immersion in a water-bath at 70°C. with continuous shaking. The contents of the tube were then transferred by suction to a 15 ml. centrifuge tube. The walls of the outer tube were washed down twice with 2 ml. portions of ethanol and the rinsings transferred to the centrifuge tube.

5 ml. of 10% calcium chloride solution were added to the contents of the centrifuge tube and mixed with gentle shaking. 2 ml. of acid-ethanol solution were then layered on to the top of the liquid in the centrifuge tube to prevent any precipitate adhering to the surface of the liquid. After standing overnight

the tube was centrifuged at 2500 r.p.m. for 15 minutes, the liquid decanted from the precipitate and the tube inverted and drained on a filter-paper. 2 ml. acid-ethanol solution were added, the precipitate broken up with a glass rod, and the rod and sides of the tube washed down with 3 ml. acid-ethanol solution. The tube was centrifuged, decanted and drained as before and residual alcohol was removed by heating the tube in a boiling water-bath for a few minutes.

1 ml. of 20% sulphuric acid was run into the tube and 0.5 ml. 1% manganese sulphate solution. The precipitate was stirred up with a pointed glass rod which was rinsed with a few drops of water. 3 ml. 0.01 N potassium permanganate solution were added and the contents of the tube stirred, washing the rod as before. After 8 to 10 minutes, 0.5 ml. 10% potassium iodide solution was added and the liberated iodine was titrated with 0.01 N sodium thiosulphate solution, a few drops of starch suspension being added as the end-point was approached. A titration blank on the reagents was carried out using water instead of the oxalate solution. If t = the titration and b the blank titration, then oxalic acid content of 24 hour urine is given by

$$0.45\ (3 - t - b) \times \frac{26}{25} \times \frac{\text{volume of 24 hour urine}}{25}$$

To test the reliability of the method a solution of potassium oxalate equivalent to 7.2 mg. oxalic acid per 100 ml. was prepared, the oxalate being dissolved in a solution containing magnesium, phosphate, sulphate and chloride in the average concentration present in urine, as suggested by Powers and Levatin. Determinations on six separate samples of this solution gave a

Table C.

Powers and Levatin method for urinary oxalate.

Recovery of oxalate added to a solution containing magnesium, phosphate, chloride and sulphate.

Oxalate added equivalent to 7.2 mg. oxalic acid per 100 ml.

	Oxalic acid found mg./100 ml.	Recovery %
1.	6.48	90.0
2.	6.53	90.8
3.	6.79	94.3
4.	6.53	90.8
5.	6.53	90.8
6.	6.62	92.1

Mean recovery 91.4 % S.D. 0.11 mg./100 ml.

percentage recovery ranging from 90.0 to 94.3 and the standard deviation from the mean was 0.11 mg. per 100 ml. (Table C).

(6) Ascorbic acid saturation tests.

The subjects for these tests were receiving ordinary hospital diet, known to be low in ascorbic acid content, throughout the test period. For three days prior to the commencement of the test and during the test period any fruit or fruit drinks which might be brought to the patients were excluded from the diet. Drugs were withheld over the same period. 500 mg. ascorbic acid were given orally each day and a 24 hour urine sample collected daily until 'saturation' was reached. An excretion of 100 mg. or more ascorbic acid in one 24 hour period was taken as indicating saturation. Urine specimens were collected in a dark bottle which contained 40 g. crushed metaphosphoric acid and the bottles were stored in a cool, dark place till the collection was complete and analysis commenced.

(7) Excretion of oxalate following intravenous ascorbic acid.

The same dietary precautions were observed during these tests as in the ascorbic acid saturation tests and, in addition, any article of food known to contain an appreciable amount of oxalate was excluded from the diet. Drugs were withheld for three days prior to, and during, the test period. 24 hour urine specimens were collected for two days and on the morning of the third day 5 g. ascorbic acid (Roche ascorbic acid for intravenous injection) was injected intravenously in a 10% solution. 24 hour urine specimens were collected on the third and fourth days and estimation of ascorbic acid and oxalic acid were carried out on each 24 hour specimen. Urine was collected in a dark bottle

containing 40 g. crushed metaphosphoric acid and 4 g. thiourea, and the bottle was stored in a dark, cool place till collection was complete. The estimation of ascorbic acid and oxalic acid was begun within an hour, or at most two hours, of completion of the collection.

RESULTS.

TABLE 1.

Time required to reach "saturation" on 500 mg. ascorbic acid per day.

	Range	Mean	S.D.
Non-rheumatoid subjects (16)	2 to 7 days	4.3 days	1.3
Rheumatoid patients (21)	3 to 8 days	5.5 days	1.4

"t" = 2.72 "p" = less than 0.05.

EXPERIMENTAL RESULTS.

(1) Ascorbic acid saturation tests.

Ascorbic acid saturation tests were carried out on a group of 21 patients with rheumatoid arthritis and on 16 control subjects. The results of the tests are given in Table 8 in the Appendix and are summarised in Table 1. There was considerable variation within each group in the number of days required to reach saturation although all the subjects were receiving the same dietary intake of ascorbic acid; in the rheumatoid arthritis group a period of from 3 to 8 days was required to reach saturation level while in the control group the time required ranged from 2 to 7 days. Although the difference between the two groups does not appear to be a large one a statistical analysis shows that it is, in fact, significant. The mean time required for saturation in the rheumatoid arthritis group was 5.5 ± 1.3 days and in the control group 4.3 ± 1.4 days. "Students" "t" test shows the difference to be significant, "t" = 2.72 and "p" less than 0.05.

To determine if changing the arbitrarily-chosen level of urinary excretion of ascorbic acid taken as indicating "saturation" made any difference to the comparison of the groups the statistical analysis was repeated taking, this time, an excretion of 50 mg. ascorbic acid in a 24 hour period as representing "saturation". In this case the difference between the two groups was still significant, "t" = 2.36 and "p" less than 0.05.

The results of the saturation tests, therefore, show that the rheumatoid arthritis patients tend to require a longer period, on the same daily oral dose of ascorbic acid, to reach saturation

TABLE 2.

Excretion of ascorbic acid after test dose of 500 mg.

A. Unsaturated subjects

	Range	Mean	S.D.
Non-rheumatoid (17)	3 to 64 mg/24 hrs.	15.8 mg.	13.8
Rheumatoid arthritis (16)	2 to 14 mg/24 hrs.	7.7 mg.	4.4

"t" = 2.24 "p" = less than 0.05

B. "Saturated" subjects

	Range	Mean	S.D.
Non-rheumatoid (11 estimations on 8 subjects)	264 to 382 mg/24 hrs.	327 mg.	45.2
Rheumatoid arthritis (31 estimations on 14 subjects)	213 to 442 mg/24 hrs.	302 mg.	59.5

"t" = 1.27 "p" = greater than 0.05.

than do normal controls.

(2) Excretion of ascorbic acid after a test dose.

In Table 2 is given a summary of the results obtained for the excretion of ascorbic acid in the 24 hour period following a single test dose of 500 mg. ascorbic acid, given orally, in a number of rheumatoid arthritis patients and non-rheumatoid controls and the full results are given in the Appendix, Table 9. The subjects included in the "unsaturated" groups had been receiving the ordinary hospital diet without vitamin supplementation for two or three weeks before the tests were made. The subjects in the "saturated" groups had received a supplement of 500 mg. ascorbic acid daily for at least eight days (the longest period which had been found necessary to reach saturation in the saturation tests) immediately prior to the test period.

When the subjects were "unsaturated" there was a significant difference between the response to the test of the two groups. The range of the excretion of ascorbic acid in the 24 hour period was 2 to 14 mg. (mean 7.7 $\pm$ 4.4 mg.) in the rheumatoid arthritis group. In the control group there was a much wider range in the results which varied from 3 to 64 mg. in the 24 hour period (mean 15.8 $\pm$ 4.4 mg.). When the subjects were saturated there was a wide variation in the results obtained in both groups, the range being 213 to 444 mg. per 24 hours (mean 302 $\pm$ 59.5 mg.) for the rheumatoid arthritis patients and 264 to 382 mg. per 24 hours (mean 327 $\pm$ 45.2 mg.) for the control subjects, and there was no statistically significant difference between the groups.

The results confirm the findings of earlier workers that

TABLE 3.

"Total" ascorbic acid content of plasma and erythrocytes.

	Range	Mean	S.D.
A. Plasma ascorbic acid.			
Non-rheumatoid (12 subjects)	0.06 to 0.58 mg. /100 ml.	0.25 mg/ 100 ml.	0.17
Rheumatoid (23 subjects)	0.03 to 0,58 mg. /100 ml.	0.22 mg/ 100 ml.	0.17
"t" = 0.50	"p" = greater than 0.1		
B. Erythrocyte ascorbic acid			
Non-rheumatoid	0.15 to 0.80 mg. /100 ml.	0.36 mg/ 100 ml.	0.18
Rheumatoid arthritis	0.21 to 1.42 mg. /100 ml.	0.54 mg/ 100 ml.	0.29
"t" = 2.05	"p" = less than 0.05		
C. Difference, erythrocyte ascorbic acid minus plasma ascorbic acid.			
Non-rheumatoid	0.05 to 0.22 mg. /100 ml.	0.10 mg/ 100 ml.	0.08
Rheumatoid arthritis	0.10 to 0.84 mg. /100 ml.	0.33 mg/ 100 ml.	0.17
"t" = 4.37	"p" = less than 0.01.		

rheumatoid arthritis patients tend to excrete less ascorbic acid than normal controls receiving the same intake but it appears that once the tissues are saturated with ascorbic acid the rheumatoid patients excrete approximately the same proportion of a test dose as do non-rheumatoid controls.

(3) Plasma ascorbic acid.

A study was made of the level of "total" ascorbic acid in a number of rheumatoid arthritis patients and non-rheumatoid controls; a summary of the results is given in Table 3, and the results are given in full in Tables 10 and 11 in the Appendix. In both groups there was considerable variation in the level of the plasma "total" ascorbic acid, the range in the rheumatoid arthritis group being 0.03 to 0.58 mg. per 100 ml. (mean 0.22 $\pm$ 0.17 mg.) and in the control group 0.06 to 0.58 mg. per 100 ml. (mean 0.25 $\pm$ 0.17 mg.). The results in the two groups do not differ significantly and the findings would appear to be at variance with those of the earlier workers. However the concentration of ascorbic acid itself was estimated in the plasma in a number of subjects in both groups (Table 12, Appendix, summarised in Table 4) and if these are compared it is seen that there is a lower concentration of ascorbic acid (and hence, presumably a higher concentration of dehydroascorbic acid + dioxogulonic acid) generally in the plasma of the rheumatoid arthritis patients than in the plasma of the control group. The ratio $\frac{\text{ascorbic acid}}{\text{total ascorbic acid}}$ was 0.36 to 0.85 (mean 0.63 $\pm$ 1.7) in the rheumatoid arthritis group and 0.61 to 0.96 (mean 0.81 $\pm$ 0.12) in the control group. Even when the intake of ascorbic acid was 500 mg. per day there was still a

TABLE 4.

Ascorbic acid and "total" ascorbic acid content of plasma.

A. Low ascorbic acid intake (50 mg. per day).

Ratio $\frac{\text{plasma ascorbic acid}}{\text{plasma "total" ascorbic acid}}$

	Range	Mean	S.D.
Non-rheumatoid (8 determinations on 4 subjects)	0.61 to 0.93	0.81	0.12
Rheumatoid arthritis (11 determinations on 5 subjects)	0.38 to 0.85	0.63	0.17

"t" = 2.56 "p" = less than 0.05.

B. High ascorbic acid intake (500 mg. per day).

Non-rheumatoid (4 determinations on 2 subjects)	0.86 to 0.99	0.95	0.06
Rheumatoid arthritis (8 determinations on 3 subjects)	0.47 to 0.87	0.73	0.15

"t" = 2.70 "p" = less than 0.05.

significant difference between the two groups the ratio $\frac{\text{ascorbic acid}}{\text{total ascorbic acid}}$ being 0.47 to 0.87 (mean 0.73 ± 0.15) for the rheumatoid group and 0.86 to 0.99 (mean 0.95 ± 0.06) for the control group. Therefore, irrespective of the amount of dietary intake of ascorbic acid there is a tendency for rheumatoid arthritis patients to have a higher plasma content of dehydroascorbic acid than control subjects receiving the same intake.

(4) Ascorbic acid content of erythrocytes.

The "total" ascorbic acid was estimated in the red blood cells of the subjects in both groups at the same time that the plasma ascorbic acid estimations were made. An estimation of the ascorbic acid content of the erythrocytes was obtained in a number of these cases in both the rheumatoid arthritis and control groups. In all these subjects, in both groups, the dehydroascorbic acid content of the erythrocytes as measured by the difference between the ascorbic acid content and "total" ascorbic acid content was negligibly small. The results are given in the Appendix, Tables 10, 11 and 13.

The estimation of "total" ascorbic acid in the erythrocytes disclosed a difference between the two groups of subjects; in the rheumatoid group the "total" ascorbic acid content of the erythrocytes was usually higher than the concentration in the plasma while in the control group this was not so. Correspondingly while there was no significant difference between the plasma "total" ascorbic acid levels in the two groups the concentration of "total" ascorbic acid in the erythrocytes was higher in the rheumatoid arthritis patients. The range was 0.22 to 1.42 mg. per 100 ml. (mean 0.54 ± 0.29 mg. per 100 ml.) in the rheumatoid group and 0.15 to 0.80 mg. per 100 ml.

TABLE 5.

Ascorbic acid content of leucocytes.

	Range	Mean	S.D.
Non-rheumatoid (8 determinations on 6 subjects)	5.0 to 20.5 mg/100 ml.	12.5mg/100 ml.	6.3
Rheumatoid arthritis (11 determinations on 10 subjects)	2.1 to 10.0 mg/100 ml.	5.9mg/100 ml.	2.7

"t" = 2.80 "p" = less than 0.05.

(mean 0.36 ± 0.18 mg. per 100 ml.) in the control group, a significant difference. If the erythrocyte - plasma difference is compared it will be seen that there is a highly significant difference between the two groups, the range of this difference (erythrocyte "total" ascorbic acid minus plasma "total" ascorbic acid) being 0.10 to 0.84 mg. per 100 ml. (mean 0.33 ± 0.17 mg. per 100 ml.) in the rheumatoid arthritis group and - 0.05 to 0.22 mg. per 100 ml. (mean 0.10 ± 0.08 mg. per 100 ml.) in the control subjects. (Table 3).

(5) "Total" ascorbic acid content of leucocytes.

In Table 5 a summary of the results obtained for the "total" ascorbic acid content of the leucocytes in ten rheumatoid arthritis patients and six control subjects is given. In the rheumatoid arthritis group the range was 2.1 to 10.0 mg. per 100 g. (mean 5.9 ± 2.7 mg. per 100 g.) and in the control group 5.0 to 20.5 mg. per 100 g. (mean 12.5 ± 6.3 mg. per 100 g.). The ascorbic acid content of the leucocytes is significantly lower in the rheumatoid group than in the control group. The full results are shown in Table 14 in the Appendix.

(6) Urinary excretion of oxalic acid.

In the period before the injection of ascorbic acid the excretion of oxalic acid was significantly lower in the rheumatoid arthritis group, a finding that is in conformity with the results obtained by Horn (1956). In eleven rheumatoid arthritis patients the excretion of oxalic acid in this period was 28.4 to 68.0 mg. per 48 hours (mean 48.4 ± 13.6 mg) and in ten experiments on nine non-rheumatoid controls it was 48.1 to 91.1 mg. per 48 hours

TABLE 6.

I. Excretion of oxalic acid.

A. Non-rheumatoid aubjects, "unsaturated" before injection of ascorbic acid (10 subjects)

Range 48.1 to 91.1 mg/48 hours. Mean 69.4 mg. S.D. 16.6

B. Rheumatoid arthritis patients, "unsaturated", before injection of ascorbic acid (11 subjects).

Range 28.4 to 68.0 mg/48 hours. Mean 48.4 mg. S.D. 13.6

C. Rheumatoid arthritis patients, "saturated", before injection of ascorbic acid (8 subjects).

Range 26.6 to 74.3 mg/48 hours. Mean 48.0 mg. S.D. 16.2

Comparison of A with B, "t" = 3.10, "p" = less than 0.01

Comparison of A with C, "t" = 2.59, "p" = less than 0.05.

II. Increase in excretion of oxalic acid in 48 hours after injection of 5 g. ascorbic acid.

A. Non-rheumatoid subjects, "unsaturated".

Range 6.1 to 95.6 mg. Mean 35.6 mg.

B. Rheumatoid arthritis patients, "unsaturated".

Range: decrease of 14.3 mg. to increase of 51.2 mg.

Mean increase 10.8 mg.

C. Rheumatoid arthritis patients, "saturated".

Range: decrease of 14.4 mg. to increase of 47.8 mg.

Mean increase 10.8 mg.

Comparison of A with B, "t" = 2.29, "p" = less than 0.05

Comparison of A with C, "t" = 2.14, "p" = less than 0.05.

(mean 69.4 ± 16.6 mg.). The increase in oxalic acid excretion in the 48 hours immediately following the intravenous injection of 5 g. ascorbic acid was also significantly lower in the rheumatoid arthritis group. In this group the results varied from a decrease of 14.3 mg. to an increase of 51.2 mg. (mean increase 10.8 mg.), the corresponding results for the control group being an increase ranging from 6.1 mg. to 95.6 mg. in 48 hours (mean increase 35.5 mg.). When the increase in the excretion of ascorbic acid in the 48 hours following the injection, compared with the 48 hours before injection, was subtracted from the dose injected and the increase in excretion of oxalic acid was calculated as a percentage of this "unaccounted for" ascorbic acid the mean percentage was 1.26 ± 2.71 for the rheumatoid group and 6.56 ± 7.03 for the control subjects. That is, of that portion of the injected ascorbic acid which is not excreted as such, or as dehydroascorbic acid or dioxogulonic acid, a smaller proportion is excreted as oxalate by rheumatoid arthritis patients (Table 6).

I am indebted to Dr. D. B. Horn for permission to include the results of one test on a rheumatoid arthritis patiant and five tests on control subjects, carried out by him, in this series.

The tests were repeated on a number of rheumatoid arthritis patients who had received 500 mg. ascorbic acid per day, orally, for nine days prior to the commencement of the test. Although these "saturated" subjects were still receiving the supplement of 500 mg. ascorbic acid daily, the range of the excretion of oxalic acid in the 48 hours before the intravenous injection of ascorbic acid was almost identical with that obtained for "unsaturated"

TABLE 7.

Excretion of ascorbic acid in 48 hours after injection of 5 g. ascorbic acid.

	Range	Mean	S.D.
Non-rheumatoid (10 subjects)	2020 to 4478 mg.	3354 mg.	760
Rheumatoid arthritis (11 subjects)	1150 to 4341 mg.	2874 mg.	930

"t" = 0.77 "p" = greater than 0.1

rheumatoid arthritis patients and again significantly lower than that obtained with "unsaturated" control subjects. The range was 26.6 to 74.3 mg. oxalic acid per 48 hours (mean 48.0 ± 16.2 mg.). The difference in excretion of oxalic acid in the 48 hours immediately following the intravenous injection of 5 g. ascorbic acid varied from a decrease of 14.4 mg. to an increase of 47.8 mg. (mean increase 10.8 mg.) per 48 hours. Again the results are very similar to those obtained in "unsaturated" rheumatoid arthritis patients and the increase in excretion of oxalate is significantly less than that obtained in "unsaturated" control subjects. The full results for the excretion of oxalic acid are given in the Appendix, Tables 15 to 17.

(7) Effect of cortisone and salicylate on blood levels, and urinary excretion, of ascorbic acid.

Since cortisone and salicylate are drugs of proved therapeutic value in the treatment of patients suffering from rheumatoid arthritis experiments were carried out to test the effect of these drugs on the levels of ascorbic acid in the plasma and erythrocytes and on the urinary excretion of ascorbic acid in rheumatoid arthritis patients and non-rheumatoid control subjects. Two rheumatoid patients and two control subjects received 50 mg. ascorbic acid daily throughout the test period while two control subjects and three rheumatoid subjects, after a preliminary period on 50 mg. per day, received 500 mg. ascorbic acid daily for the remainder of the test period. One of the rheumatoid patients on the low ascorbic acid intake and one on the high intake was given cortisone while the other rheumatoid arthritis

subjects were given salicylate, both drugs being given in ordinary therapeutic doses. In each pair of control subjects receiving the same supplement of ascorbic acid one subject was given cortisone and one salicylate. Daily 24 hour collections of urine were obtained throughout the test period for the estimation of ascorbic acid and dehydroascorbic acid. Blood was withdrawn twice during each sub-section of the test period, usually of five days duration, for the estimation of ascorbic acid and "total" ascorbic acid in the plasma and erythrocytes.

The results are shown in Tables 18 to 26 in the Appendix. In the subjects who received a low ascorbic acid intake neither cortisone nor salicylate appeared to have any clearly-defined effect on the concentration of ascorbic acid and dehydroascorbic acid present in the plasma or erythrocytes, or on the urinary excretion. The rheumatoid arthritis patients who received the high ascorbic acid intake showed a marked rise in both the plasma and erythrocyte levels of ascorbic acid and in the amount excreted in the urine immediately following the start of the cortisone or salicylate therapy. The two control subjects who received a high intake of ascorbic acid had reached "saturation" level before the cortisone or salicylate dosage was commenced and the drugs appeared to produce no appreciable change in the concentration of ascorbic acid in plasma or erythrocytes.

In both these subjects the excretion of ascorbic acid in the 24 hour urine increased to over 500 mg. in the first period after commencement of dosage with the drug but this effect was not maintained. In the rheumatoid arthritis patients the

concentration of plasma dehydroascorbic acid, which was generally higher than in the non-rheumatoid subjects, appeared to be reduced as a result of the cortisone or salicylate therapy.

In order to investigate further the effects of cortisone and salicylate on blood levels and urinary excretion of ascorbic acid in rheumatoid arthritis patients experiments were carried out over a much longer period on a few subjects. These patients received ordinary hospital diet without vitamin supplement or drugs for two weeks; during the following two weeks 500 mg. ascorbic acid was given orally every day and during a third period of two weeks cortisone or salicylate (in normal therapeutic doses) was given in addition to 500 mg. ascorbic acid per day. This period on one or other of the drugs was followed by two weeks on 500 mg. ascorbic acid daily but with no drugs and, finally, by a two week period on hospital diet without ascorbic acid supplement or drugs. 24 hour urine specimens were collected every second day throughout the test period of ten weeks and blood samples were withdrawn at seven day intervals for estimation of "total" ascorbic acid in plasma and erythrocytes.

The results obtained from these experiments are shown in the Appendix, Tables 27 to 32 and suggest that in rheumatoid arthritis patients, once "saturation" level has been obtained, cortisone and salicylate cause no change in the plasma or erythrocyte level of ascorbic acid. In some of the subjects there was evidence of an increased urinary output of ascorbic acid immediately following commencement of dosage with cortisone or salicylate but when this did occur it appeared to be transitory.

DISCUSSION.

DISCUSSION.

The experimental results confirm the findings of Rinehart and his associates and the other earlier workers that in general there is a lower concentration of ascorbic acid in the plasma of rheumatoid arthritis patients, and a lower excretion of the vitamin in the urine, than in non-rheumatoid subjects, receiving the same amount of dietary ascorbic acid. The results obtained in the ascorbic acid saturation tests support the view that there exists a deficiency of ascorbic acid, or an increased requirement for the vitamin, in the rheumatoid condition. Further differences between rheumatoid arthritis patients and normal controls have been demonstrated in the course of the investigation; it has been shown that rheumatoid arthritis patients have a higher concentration of dehydroascorbic acid in the plasma and a higher concentration of ascorbic acid in the erythrocytes than normal controls; that they have a lower concentration of "total" ascorbic acid in the leucocytes, and that they excrete less oxalate in the urine than normal subjects when the intake of ascorbic acid is the same. The question arises therefore as to how far this new data contributes to providing an explanation of the apparent deficiency of ascorbic acid in the rheumatoid condition.

Of the possible explanations which might be advanced for this apparent deficiency those most worth consideration would appear to be (a) a poor dietary intake, (b) a defect in intestinal absorption in rheumatoid arthritis patients, (c) an alteration in the rate of urinary excretion of ascorbic acid, (d) some alteration in the

body storage and (e) an alteration in the metabolism of the vitamin which might involve either increased destruction, whether useful or wasteful, or an altered pathway of metabolism.

A point that has been advanced in favour of the view that the deficiency is due to a poor dietary intake is that there is an almost complete absence of rheumatoid arthritis in the Tropics where true clinical scurvy is practically unknown. However there appears to be some doubt as to the truth of the claim concerning the absence of rheumatoid arthritis in the Tropics and, in any case, in view of the different climatic conditions any difference in the incidence of the disease between the Tropics and other zones can not be attributed solely to dietary factors. Under present conditions true clinical scurvy is a very rare condition in this country and there is no evidence that the incidence of rheumatoid arthritis is confined to those social classes in which scurvy does occasionally occur. On the contrary scientifically conducted surveys in both Europe and America have shown that the disease occurs in all social groups and in widely differing climatic conditions. It seems unlikely therefore that dietary intake can play any important part in the causation of the disease or of the deficiency in ascorbic acid which appears to accompany it. This view is supported by the work of Bayles, Richardson and Hall (1943) who examined the nutritional background of thirty-one patients with rheumatoid arthritis; they found that only eight had an intake of below 25 mg. per day and sixteen had an intake of over 50 mg. per day - up to as high as 160 mg. per day - and they concluded that there was no evidence that a previous dietary

deficiency played any part in the aetiology of rheumatoid arthritis.

The lower plasma levels and decreased urinary excretion of ascorbic acid in rheumatoid arthritis patients and the longer time required to reach saturation on a standard daily dose of the vitamin could be explained, given similar intakes, by a diminished rate of intestinal absorption. Such a defect in absorption might have explained the lower excretion of oxalic acid in rheumatoid arthritis reported by Horn (1956) and confirmed in the present investigation, but rheumatoid arthritis patients showed a smaller increase in oxalate excretion after intravenous doses of ascorbic acid (Table 6) and in this case intestinal absorption was not involved. When the "total" plasma ascorbic acid was measured (Table 3) there was no difference between the rheumatoid and control groups while in the erythrocytes the level of ascorbic acid was actually higher in the rheumatoid arthritis patients than in the controls when both groups received the same intake of ascorbic acid. (Table 3). It is difficult to reconcile these findings with a diminished rate of intestinal absorption of ascorbic acid.

A diminished rate of renal excretion of ascorbic acid could be the explanation of the lower excretion of ascorbic acid observed in rheumatoid arthritis patients and for the longer time required to reach an arbitrarily chosen level of excretion in the ascorbic acid saturation tests. Ralli, Friedman and Rubin (1938) found that the excretion of ascorbic acid was governed by (1) the plasma level, (2) the rate of glomerular filtration and (3) the maximal rate of tubular re-absorption, so that a diminished

excretion might arise because of a defect in glomerular filtration or an abnormally high rate of tubular re-absorption. It would be possible to obtain figures ostensibly representing the rate of glomerular filtration and the rate of tubular re-absorption in rheumatoid arthritis patients and control subjects but, owing to the labile nature of ascorbic acid and dehydroascorbic acid. such measurements would be of little value. There could be no means of taking into account any interconversion of ascorbic acid and dehydroascorbic acid, or of any destruction of ascorbic acid via dioxogulonic acid, in the tubules or in the cells of the tubules. However, it is worthy of note that when rheumatoid and control subjects had been brought to a state of "saturation" with large doses of ascorbic acid there was no significant difference in the 24 hour excretion after a test dose between the two groups (Table 2). Similarly, there was no significant difference between rheumatoid arthritis and control subjects in the amount of ascorbic acid excreted in the 48 hours after the intravenous injection of 5 g. ascorbic acid (Table 7), when very large amounts - up to 4 g. in 24 hours - were excreted. It does not appear therefore that there is any inability to excrete ascorbic acid on the part of the rheumatoid arthritis patient.

An attempt to explain the apparent deficiency of ascorbic acid observed in rheumatoid arthritis on the basis of dietary intake, diminished intestinal absorption or a decreased rate of urinary excretion would still leave the new facts brought to light in the present investigation. None of these theories would explain the higher concentration of dehydroascorbic acid found

in the plasma or the higher concentration of ascorbic acid in the erythrocytes of rheumatoid arthritis patients and it appears unlikely that these differences between rheumatoid arthritis and normal subjects should be unrelated to the difference in plasma ascorbic acid level and urinary excretion between the groups.

Booker et al (1951) found evidence of a barrier between plasma and cells towards ascorbic acid, but their methods estimated ascorbic acid only and did not take account of dehydroascorbic acid. From the work of Lloyd (1951), Golden and Sargent (1952) and Lloyd and Parry (1954), and confirmed by Iggo, Stewart and Thomson (1958), it is now known that the cell wall is readily permeable to dehydroascorbic acid but that ascorbic acid passes through it only slowly. It has been found (Stewart, Horn and Robson, personal communication), that dehydroascorbic acid is not produced, to any appreciable extent, in the plasma and hence the higher concentration of dehydroascorbic acid in the plasma must be due to a tissue process. Iggo, Thomson and Stewart (1958) have shown that the erythrocytes of rheumatoid arthritis patients reduce dehydroascorbic acid more readily than do those of non-rheumatoid controls. Thus, as a consequence of increased oxidation of ascorbic acid to dehydroascorbic acid in the rheumatoid patient, dehydroascorbic acid would be expected to pass into the erythrocytes at a greater rate and there to be reduced to ascorbic acid which will be retained within the cell membrane; this is the picture actually found.

An increased intake of dehydroascorbic acid into the cells and its conversion there to ascorbic acid leading to an increased concentration of ascorbic acid in the cell suggests the possibility

that the lowered plasma levels and decreased urinary excretion of the vitamin in rheumatoid arthritis may be due to an increased tissue storage. It is not known however if this phenomena of reduction of dehydroascorbic acid takes place in cells other than the erythrocytes. Since it has been demonstrated by Crandon and his co-workers and others that the appearance of symptoms of scurvy in prolonged ascorbic acid deprivation is heralded by the virtual disappearance of ascorbic acid from the leucocytes the concentration in the leucocytes has generally been taken as providing the best indication - short of the analysis of biopsy material from various organs - of the state of body storage. The ascorbic acid content of the leucocytes was lower in the rheumatoid arthritis group than in the control subjects which would suggest a lower than normal body store.

If, however, there is no difference in intake, absorption or excretion of ascorbic acid the explanation of a lower body store remains to be found and the answer must surely lie in an alteration of metabolism. The higher concentration of ascorbic acid in the erythrocytes can be understood as a consequence of the higher plasma concentration of dehydroascorbic acid since not only must there be an unusually rapid passage of dehydroascorbic acid into the erythrocytes, but there is unusually rapid regeneration of it into ascorbic acid within the erythrocytes. This, in turn, argues an increased rate of oxidation of ascorbic acid to dehydroascorbic acid which, as has been shown, is a tissue process, or some inhibition of its further oxidation to dioxogulonic acid. An increased rate of interconversion of ascorbic acid and dehydro-

ascorbic acid need not necessarily affect the net loss by oxidation but it could affect it in either direction.

The lower rate of excretion of oxalic acid found in rheumatoid arthritis patients is not due to diminished intake of ascorbic acid or to poor absorption of the vitamin since it is apparent even when rheumatoid arthritis patients are brought to "saturation" with ascorbic acid and also when the ascorbic acid is given by the intravenous route. This diminished urinary excretion of oxalate would therefore appear to indicate a difference in the metabolic pathway of ascorbic acid. It might be taken as evidence of a diminished net rate of oxidation of ascorbic acid but until more is known about the normal metabolic pathway of ascorbic acid in man this cannot be assumed.

Damron, Monier and Roe (1952) did not consider that the reactions ascorbic acid $\rightleftharpoons$ dehydroascorbic acid $\rightarrow$ dioxogulonic acid represented the normal metabolic pathway in animal tissues. On the other hand the work of Curtin and King (1955) and Burns, Dayton and Schulenberg (1956), which has already been discussed, would appear to support the view that, in the guinea-pig and rat at least, the main pathway of metabolism of ascorbic acid is via dehydroascorbic acid to dioxogulonic acid and thence by oxidation of the entire carbon chain to carbon dioxide. Curtin and King found that when they injected "labelled" ascorbic acid, dehydro-ascorbic acid and dioxogulonic acid the proportion of the radio-activity found in the respiratory carbon dioxide and also in the urinary oxalate was least when ascorbic acid was injected and greatest when dioxogulonic acid was injected. It would appear

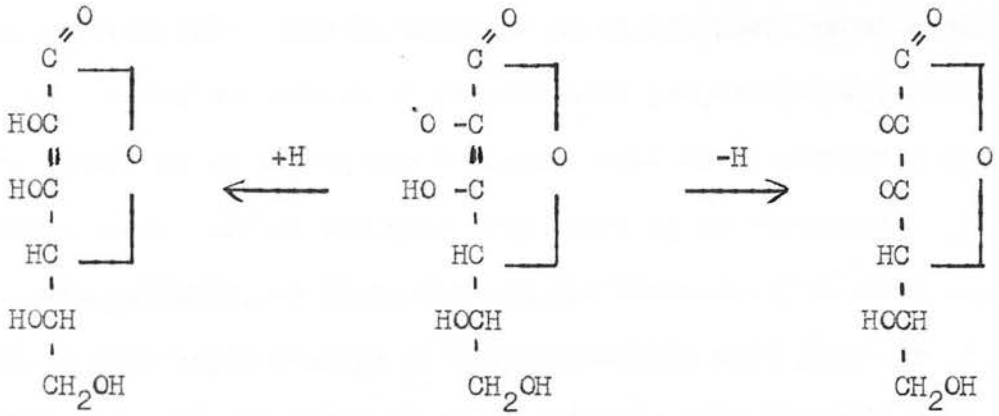
probable therefore that both the carbon dioxide and the oxalate arise after conversion to dioxogulonic acid but the experiments of Curtin and King and of Burns, Dayton and Schulenberg give no information as to whether prodution of oxalic acid is a necessary intermediate step in the conversion of dioxogulonic acid to carbon dioxide or whether it represents a divergent pathway.

The results of Hellman and Burns (1955) who injected L-ascorbic-1-C^{14} acid into two human subjects are somewhat at variance with those obtained by Curtin and King and Burns, Dayton and Schulenberg with animals since they found that only 10% of the injected radio-activity appeared as respiratory $C^{14}O_2$ and approximately 40% was excreted as oxalate. The difference might be due to a difference in the relation of the dose given to the biological requirement of the species. There are conflicting views as to human requirement for ascorbic acid but there is evidence that it may be extremely low; Bartley and his associates (1953) reported that 10 mg. ascorbic acid per day was sufficient to cure frank human scurvy completely while volunteers remained free from scurvy for over six months while receiving daily intakes which averaged less than 5 mg.

Damron and his co-workers found that a small proportion only of the "total" ascorbic acid in all tissues was present as dehydro-ascorbic acid and dioxogulonic acid. It has long been considered probable that ascorbic acid functions as a hydrogen acceptor in some biological oxidation system but Meiklejohn (1953) has pointed out that it is dehydroascorbic acid which could function as a hydrogen acceptor and he has put forward the suggestion that the

ascorbic acid in the body tissues serves as a source of a small, but continuous, supply of dehydroascorbic acid. Neuberger (1949), in discussing the mechanism of the oxidation of tyrosine suggested that the oxidation of tyrosine or its keto-acids to homogentisic acid involves an attack by an electrophilic reagent. Weissberger and Lu Valle (1944), who studied the copper-catalysed auto-oxidation of ascorbic acid in vitro, concluded from their results that a monovalent ion is the substrate of the copper catalyst; regarding the monovalent ascorbate ion as the substrate they consider Cu^{++} as an electron acceptor which can co-ordinate with the substrate before an electron shift takes place, giving an "ascorbic semiquinone", which might perhaps be formulated as a free radicle. Cu^{+} which can co-ordinate less readily is released and oxidised by oxygen. Lloyd and Sinclair (1953) suggest that this semiquinone, which they take to be identical with the monodehydroascorbic acid of other writers, is the electrophilic (catanoid) reagent postulated by Neuberger (1949), in view of the evidence that ascorbic acid is necessary for the hydroxylation of p-hydroxy-phenylpyruvic acid to 2:5 dihydroxyphenylpyruvic acid.

Definite chemical evidence for the existence of monodehydro-ascorbic acid has not been obtained; it is a hypothetical intermediate in a number of oxidations in which ascorbic acid appears to intervene in vitro and in vivo. Such a catanoid radicle could function as a hydrogen acceptor or a hydrogen donator.



L-ascorbic acid catanoid radicle dehydroascorbic acid

Lloyd and Sinclair (1953) point out that it may be argued that the concentration of monodehydroascorbic acid will be proportional to the products of the concentrations of ascorbic acid and dehydroascorbic acid and that, for a given concentration of total ascorbic acid, the maximum concentration of monodehydroascorbic acid will be found at 50% oxidation to dehydroascorbic acid. The fact that it has been found that there is a tendency for rheumatoid arthritis patients to have a higher concentration of plasma dehydroascorbic acid than control subjects on the same ascorbic acid intake may, therefore, be an indication of an increased requirement for the production of monodehydroascorbic acid. If this be so it would be expected that this higher plasma concentration of dehydroascorbic acid would result in an increased rate of conversion to dioxogulonic acid and hence to an increased production of oxalic acid; yet Horne (1956) found that the tendency was for rheumatoid arthritis patients to excrete less oxalic acid than normal controls under the same dietary conditions and his findings have been confirmed in the present investigation.

If it be postulated that there is increased production of

monodehydroascorbic acid in response to an increased requirement for some biological oxidation process then the monodehydroascorbic acid, in acting as a hydrogen acceptor, will be re-converted to ascorbic acid. If at the same time there is in rheumatoid arthritis patients, as Iggo, Stewart and Thomson (1958) have shown, a more rapid passage of dehydroascorbic acid into the erythrocytes and reduction there to ascorbic acid it could be that this more rapid turnover of dehydroascorbic acid to ascorbic acid would result in a decreased tendency for dehydroascorbic acid to be converted to dioxogulonic acid and hence to a reduced production of oxalic acid.

If, on the other hand, the maintenance of a higher concentration of dehydroascorbic acid - necessary to maintain an increased supply of monodehydroascorbic acid - results in an increased production of dioxogulonic acid and this in turn results in an increased production of oxalic acid the lower urinary excretion of oxalate in the rheumatoid patient must be due to a difference in the metabolism of oxalate. If the oxalate derived from ascorbic acid which is excreted in the urine represents an escape of oxalate from some tissue enzyme system then this tendency in rheumatoid arthritis to excrete less oxalate could be a result of increased tissue oxidation of oxalate to carbon dioxide. On the other hand, since at present there is not sufficient evidence to say whether dioxogulonic acid can be oxidised directly to carbon dioxide in the body without the intermediate formation of oxalic acid, this tendency to excrete less oxalic acid may be due to an increased rate of direct oxidation of dioxogulonic acid to carbon

dioxide in the rheumatoid condition.

The role of ascorbic acid in tyrosine metabolism is the only part of the vitamin's activity in which the molecular processes that depend upon ascorbic acid are fairly clearly understood, although the work of Gould and Woessner (1957) is evidence of another possible role - in the hydroxylation of proline to hydroxyproline in collagen formation. It cannot be assumed that ascorbic acid may not be necessary for other metabolic processes within the animal organism and until more information is obtained regarding the functions and metabolic fate of ascorbic acid in man no clear explanation of the observed differences between rheumatoid arthritis patients and non-rheumatoid controls can be advanced.

It would be of assistance in considering possible explanations of the tendency of rheumatoid arthritis patients to excrete less oxalate in the urine if it were known whether there is any difference between rheumatoid arthritis patients and non-rheumatoid controls in the proportion of ascorbic acid, dehydroascorbic acid and dioxogulonic acid converted to respiratory carbon dioxide when each of these substances is injected. This data could best be obtained by experiments using ascorbic acid "labelled" with radio-active carbon - experiments outwith the scope of the present investigation. Without such information all that can be stated definitely is that the results obtained support the view that the apparent deficiency of ascorbic acid which has been observed in rheumatoid arthritis patients is due to an alteration in the metabolism of the vitamin and is not due simply to a difference in intake, absorption, excretion or storage of ascorbic acid.

Schroeder (1935), Harde et al (1935), Bullowa and co-workers (1936) and other workers have shown that a deficiency state with regard to ascorbic acid exists in a number of infectious conditions. The question arises therefore as to whether the apparent alteration in the metabolism of ascorbic acid is due directly to the rheumatoid arthritis disease process or whether it is a non-specific response to an inflammatory condition. Roe, Kuether and Zimler (1947) determined the levels of total ascorbic acid in the plasma and whole blood of 50 patients with various infectious conditions and found that in every case the concentration of total ascorbic acid was lower in the plasma than in whole blood; the concentration in the erythrocytes must therefore have been greater than in the plasma in each case. On the other hand Iggo et al (1958) found that although the erythrocytes of patients suffering from tuberculosis tended to reduce dehydroascorbic acid to ascorbic acid at a slightly more rapid rate than did the erythrocytes of control subjects in only one tuberculosis patient was the reducing-power of the erythrocytes above the upper limit of the normal range. The erythrocytes from rheumatoid arthritis patients had a reducing-power for dehydroascorbic acid that was significantly higher than that of the erythrocytes from control subjects.

There is no information available with regard to the urinary excretion of oxalate in infectious conditions. Further investigation is required to compare (1) plasma and erythrocyte levels of ascorbic acid on the same dietary intake of the vitamin, (2) the ability of the erythrocytes to reduce dehydroascorbic acid to ascorbic acid, and (3) the urinary excretion of oxalate under similar dietary

conditions, in rheumatoid arthritis patients, patients with various infectious conditions and normal controls. One difficulty in making such a comparison would be to obtain satisfactory objective criteria to assess the degree of inflammation present.

The results of the experiments in which cortisone or salicylate was given are difficult to assess. There was a transitory rise in the urinary excretion of ascorbic acid which occurred after the commencement of dosage with either of the drugs; increases in the urinary excretion of ascorbic acid have been reported as a result of dosage with salicylate (Spitzer and Shapiro, 1948) and the effect observed could be a purely renal one. However, the two rheumatoid arthritis patients who were receiving a high ascorbic acid intake showed a marked rise in the plasma level of ascorbic acid also immediately after commencement of the drug and it is difficult to conceive an alteration in the excretory mechanism which could produce a rise in plasma concentration at the same time as an increase in urinary excretion. A more probable explanation is that the coincident rise in plasma concentration and increased urinary excretion of ascorbic acid is due to an action of the drug in causing the ascorbic acid to pass from storage in the body tissues to circulation in the blood. It would seem probable however that the two control subjects on whom this particular experiment was performed, and whose initial plasma ascorbic acid concentration was considerably higher, would have a store in the tissues at least as great as the rheumatoid arthritis subjects, yet the effect of the drugs on the plasma level and urinary excretion in these control subjects was much less marked. It

could be, however, that the effect of cortisone or salicylate on the disease-process present in the rheumatoid arthritis patients was such as to correct the alteration in ascorbic acid metabolism and thus - perhaps by a reduction in the rate of oxidation - to have an "ascorbic acid sparing" effect, but there is as yet not sufficient evidence to decide between these possibilities.

SUMMARY.

SUMMARY.

(1) It has been confirmed that rheumatoid arthritis patients have a lower plasma level and a lower urinary excretion of ascorbic acid than non-rheumatoid controls receiving the same intake of ascorbic acid.

(2) It has been shown that these patients require a longer time to reach saturation with the vitamin, on a standard daily dose, than control subjects.

(3) It has been shown also that rheumatoid arthritis patients have a lower level of ascorbic acid in the leucocytes, a higher dehydroascorbic acid concentration in the plasma and a higher ascorbic acid concentration in the erythrocytes than do normal subjects on the same dietary intake.

(4) Patients with rheumatoid arthritis excrete less oxalic acid than do non-rheumatoid controls and show a smaller increase in excretion of oxalic acid after a large intravenous dose of ascorbic acid.

(5) The implication of these findings has been discussed and points requiring further investigation have been suggested.

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APPENDIX.

In the Tables which follow, the following abbreviations are used:

AA = ascorbic acid

DHA = dehydroascorbic acid

TAA = "total" ascorbic acid
(i.e. ascorbic acid + dehydroascorbic acid + dioxogulonic acid).

OA = oxalic acid.

TABLE 8.

Ascorbic Acid Saturation Tests.

Non-rheumatoid subjects		Rheumatoid arthritis subjects	
Subject No.	Number of days to reach saturation.	Subject No.	Number of days to reach saturation.
(1)	7	(1)	5
(2)	4	(2)	6
(3)	3	(3)	7
(4)	3	(4)	5
(5)	7	(5)	4
(6)	5	(6)	6
(7)	4	(7)	5
(8)	3	(8)	5
(9)	2	(9)	8
(10)	3	(10)	7
(11)	4	(11)	8
(12)	4	(12)	6
(13)	5	(13)	5
(14)	5	(14)	3
(15)	4	(15)	4
(16)	5	(16)	6
		(17)	4
		(18)	4
		(19)	6
		(20)	6
		(21)	6
Mean 4.3 days		Mean 5.5 days	
S.D. 1.4		S.D. 1.3	

"t" = 2.72

"p" = less than 0.05

TABLE 9.

Excretion of Ascorbic Acid in 24 hour period after test dose (500 mg.)

A. "Unsaturated" subjects.

Non-rheumatoid subjects.		Rheumatoid arthritis subjects.	
No.	mg. TAA per 24 hours	No.	mg. TAA per 24 hours.
(1)	12	(1)	14
(2)	6	(2)	12
(3)	6	(3)	12
(4)	6	(4)	6
(5)	4	(5)	6
(6)	7	(6)	7
(7)	6	(7)	14
(8)	9	(8)	10
(9)	12	(9)	9
(10)	64	(10)	3
(11)	17	(11)	7
(12)	32	(12)	2
(13)	37	(13)	4
(14)	3	(14)	2
(15)	11	(15)	2
(16)	8	(16)	13
(17)	28		
Mean 15.8 mg.		Mean 7.7 mg.	
S.D. 13.8		S.D. 4.4	

"t" = 2.24

"p" = less than 0.05

Table 9 (continued)

B. "Saturated" subjects.

Non-rheumatoid subjects		Rheumatoid arthritis subjects	
No.	mg. TAA per 24 hours	No.	mg. TAA per 24 hours.
(1)	374	(1)	306
	264		300
			320
(2)	323	(2)	405
	382		348
(3)	379		430
(4)	280	(3)	444
(5)	307		428
(6)	334		235
(7)	373	(4)	256
(8)	268		378
	312		332
		(5)	264
Mean 327 mg.			274
S.D. 45.2		(6)	292
			260
			300
		(7)	294
			324
			278
		(8)	239
			213
		(9)	281
			250
		(10)	257
			232
		(11)	299
		(12)	254
		(13)	293
		(14)	283
			298
		Mean 302 mg.	
		S.D. 59.5	

"t" = 1.27

"p" = greater than 0.05

TABLE 10.

Concentration of Total Ascorbic Acid in Plasma and Erythrocytes.

Non-rheumatoid subjects.

No.	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Ratio $\frac{\text{Plasma TAA}}{\text{Erythrocyte TAA}}$	Difference: Erythrocyte TAA - plasma TAA mg./100 ml.
(1)	0.06	0.20	0.33	0.14
(2)	0.20	0.15	1.33	- 0.05
(3)	0.32	0.37	0.86	0.05
(4)	0.58	0.54	1.07	- 0.04
(5)	0.07	0.19	0.37	0.12
(6)	0.13	0.30	0.43	0.17
(7)	0.58	0.80	0.73	0.22
(8)	0.30	0.45	0.67	0.15
(9)	0.20	0.32	0.63	0.12
(10)	0.27	0.41	0.66	0.14
(11)	0.12	0.20	0.60	0.08
(12)	0.21	0.34	0.62	0.13
	Mean 0.25 mg./100 ml.	Mean 0.36 mg./100 ml.	Mean 0.69	Mean 0.10 mg./100 ml.
	S.D. 0.17	S.D. 0.18	S.D. 0.29	S.D. 0.08

TABLE 11.

Concentration of Ascorbic acid and Total Ascorbic acid in Plasma and Erythrocytes.

Rheumatoid arthritis subjects.

No.	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Ratio $\frac{\text{Plasma TAA}}{\text{Erythrocyte TAA}}$	Difference: Erythrocyte TAA - plasma TAA mg./100 ml.
(1)	0.17	0.54	0.32	0.37
(2)	0.03	0.46	0.07	0.43
(3)	0.08	0.23	0.35	0.15
(4)	0.58	1.04	0.59	0.46
(5)	0.32	0.42	0.76	0.10
(6)	0.58	1.42	0.41	0.84
(7)	0.52	0.87	0.60	0.35
(8)	0.33	0.43	0.77	0.10
(9)	0.26	0.75	0.35	0.49
(10)	0.38	0.90	0.42	0.52
(11)	0.23	0.57	0.40	0.34
(12)	0.20	0.44	0.45	0.24
(13)	0.20	0.56	0.36	0.36
(14)	0.16	0.65	0.25	0.49
(15)	0.06	0.50	0.12	0.44
(16)	0.19	0.40	0.48	0.21
(17)	0.15	0.51	0.29	0.36
(18)	0.04	0.21	0.19	0.17
(19)	0.20	0.43	0.47	0.23
(20)	0.13	0.28	0.46	0.15
(21)	0.06	0.48	0.13	0.42
(22)	0.05	0.22	0.23	0.17
(23)	0.11	0.22	0.50	0.11
	Mean 0.22 mg./100 ml.	Mean 0.54 mg./100 ml.	Mean 0.39	Mean 0.33 mg./100 ml.
	S.D. 0.17	S.D. 0.29	S.D. 0.18	S.D. 0.17
	"t" = 0.50	"t" = 2.05	"t" = 3.75	"t" = 4.37
	"p" = greater than 0.1	"p" = less than 0.05	"p" = less than 0.01	"p" = less than 0.01

TABLE 12.

Ascorbic Acid and Total Ascorbic Acid Content of Plasma.

A. Low ascorbic acid intake (50 mg. per day).

Non-rheumatoid subjects.

No.	Plasma AA mg./100 ml.	Plasma TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$
(1)	0.98	1.08	0.93
(2)	0.78	1.05	0.74
(3)	1.03	1.07	0.96
(4)	0.89	1.00	0.89
(5)	0.45	0.74	0.61
(6)	0.69	0.78	0.88
(7)	0.50	0.70	0.71
(8)	0.43	0.55	0.79
			Mean 0.81
			S.D. 0.12

Rheumatoid arthritis subjects.

(1)	0.22	0.26	0.85
(2)	0.25	0.30	0.83
(3)	0.12	0.32	0.38
(4)	0.21	0.30	0.70
(5)	0.19	0.38	0.50
(6)	0.28	0.33	0.85
(7)	0.42	0.82	0.52
(8)	0.43	0.58	0.74
(9)	0.31	0.64	0.49
(10)	0.27	0.52	0.52
(11)	0.32	0.58	0.54
			Mean 0.63
			S.D. 0.17

"t" = 2.56 "p" = less than 0.05.

B. High ascorbic acid intake (500 mg. per day).

Non-rheumatoid subjects.

(1)	0.91	1.05	0.86
(2)	1.40	1.43	0.98
(3)	1.89	1.90	0.99
(4)	1.95	2.00	0.98
			Mean 0.95
			S.D. 0.06

Rheumatoid arthritis subjects.

(1)	0.43	0.58	0.74
(2)	0.65	0.76	0.86
(3)	0.22	0.48	0.47
(4)	0.56	0.74	0.76
(5)	0.24	0.30	0.80
(6)	0.13	0.25	0.52
(7)	0.55	0.63	0.87
(8)	0.95	1.13	0.84
			Mean 0.73
			S.D. 0.15

"t" = 2.70 "p" = less than 0.05.

TABLE 13.

Concentration of Ascorbic Acid and Total Ascorbic Acid in Erythrocytes.

Non-rheumatoid subjects.

No.	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$
(1)	0.59	0.70	0.84
	0.95	0.96	0.99
(2)	0.70	0.73	0.96
	0.76	0.80	0.95
(3)	1.07	1.10	0.97
	1.10	1.10	1.00
(4)	0.99	1.01	0.98
	0.97	1.00	0.97
			Mean 0.96
			S.D. 0.04

Rheumatoid arthritis subjects.

No.	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$
(1)	0.36	0.42	0.86
	0.60	0.67	0.90
(2)	0.84	0.90	0.93
	0.44	0.43	1.02
(3)	0.98	1.04	0.94
	0.90	0.93	0.97
(4)	1.22	1.42	0.86
			Mean 0.93
			S.D. 0.05

"t" = 1.44 "p" = greater than 0.1

TABLE 14.

Total Ascorbic Acid Content of Leucocytes.

Non-rheumatoid subjects.		Rheumatoid arthritis subjects.	
No.	TAA mg./100 ml.	No.	TAA mg./100 ml.
(1)	7.7	(1)	2.1
	5.0	(2)	7.0
(2)	17.6	(3)	8.5
	20.5	(4)	2.6
(3)	14.0	(5)	9.0
(4)	19.9	(6)	6.5
(5)	8.0	(7)	2.7
(6)	7.1	(8)	5.8
		(9)	10.0
		(10)	4.1
		(11)	6.4
Mean 12.5 mg./100 ml.		Mean 5.9 mg./100 ml.	
S.D. 6.3		S.D. 2.7	

"t" = 2.80

"p" = less than 0.05

TABLE 15.

Urinary excretion of total ascorbic acid and oxalic acid.

5 g. ascorbic acid injected intravenously.

Non-rheumatoid subjects.

No.	Before injection Urine TAA mg/48 hours	Urine OA mg/48 hours	After injection Urine TAA mg/48 hours	Urine OA mg/48 hours	Increase in OA mg/48 hours	Increase in OA calculated as percentage of "unaccounted for" AA.
(1)	27	80.3	3719	86.4	6.1	0.47
(2)	11	48.1	4478	97.3	49.2	18.05
(3)	10	51.3	4070	146.9	95.6	19.88
(4)	45	84.5	3611	98.8	14.3	2.36
(5)	34	91.1	3109	108.4	17.3	1.24
(6)a	27	83.9	2924	146.4	62.5	6.89
(6)b	19	53.9	2926	95.3	41.4	4.51
(7)	25	72.7	2020	87.8	15.1	1.64
(8)	21	57.4	2606	70.1	12.7	2.04
(9)	14	35.9	4078	76.6	40.7	8.52
Mean	23	69.4	3354	-	35.5	6.56
S.D.	12	16.6	760	-		7.03

TABLE 16.

Urinary Excretion of Ascorbic Acid and Oxalic Acid.

Rheumatoid arthritis subjects.

No.	Before injection Urine TAA mg/48 hours	Urine OA mg/48 hours	After injection Urine TAA mg/48 hours	Urine OA mg/48 hours	Increase in OA mg/48 hours	Increase in OA as percentage of "unaccounted for" AA.
(1)	5	35.2	1150	55.6	20.4	1.01
(2)	8	37.9	2817	35.7	- 2.2	no increase
(3)	12	28.4	3373	22.5	- 5.9	no increase
(4)	21	50.7	1709	65.1	14.4	0.26
(5)	20	54.1	1906	105.3	51.2	3.29
(6)	14	68.4	3439	94.0	25.6	3.19
(7)	8	55.7	4341	42.0	-13.7	no increase
(8)	10	52.0	3412	37.7	-14.3	no increase
(9)	17	53.7	3436	89.5	35.8	4.44
(10)	15	30.3	3253	22.9	- 7.4	no increase
(11)	15	66.1	2779	81.3	15.2	1.63
Mean	13	48.4	2874	-	10.8	1.26
S.D.	5.1	13.6	930	-		2.71
"t"	3.04	3.10	0.77	-	2.29	2.43
"p"	less than 0.01	less than 0.01	greater than 0.10	-	less than 0.05	less than 0.05

TABLE 17.

Urinary Excretion of Oxalic Acid in Rheumatoid Arthritis Patients after "Saturation" with Ascorbic Acid.

No.	Before injection OA mg./48 hours	After injection OA mg./48 hours	Increase in OA mg./48 hours.
(1)	50.4	40.4	- 10.0
(2)	74.3	82.6	8.3
(3)	52.5	60.2	7.7
(4)	40.3	46.6	6.3
(5)	66.3	114.1	47.8
(6)	34.7	44.3	9.6
(7)	26.6	58.4	31.8
(8)	38.7	24.3	- 14.4
Mean	48.0	-	10.8
S.D.	13.6	-	-
Compared with "unsaturated" control subjects)	"t" = 2.59	-	"t" = 2.14
	"p" = less than 0.05	-	"p" = less than 0.05.

TABLE 18.

Effect of salicylate. Non-rheumatoid subject No. 1.

	PLASMA			ERYTHROCYTES			URINE			
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	AA + DHA mg./24 hrs.	Ratio $\frac{AA}{AA + DHA}$	Period
1	0.45	0.74	0.61	0.59	0.70	0.84	30	38	0.84	
2							20	28	0.72	Preliminar
3	0.69	0.78	0.88	0.95	0.96	0.99	18	29	0.62	
4							20	28	0.72	
5	0.61	0.71	0.86	0.68	0.70	0.97	27	33	0.82	Salicylat
6							22	27	0.82	
7	0.61	0.79	0.77	0.84	0.87	0.97	23	29	0.79	
8							21	29	0.72	
9							21	25	0.84	Reducing
10	0.76	0.84	0.91	0.81	0.85	0.95	35	40	0.88	doses of
11							20	23	0.87	Salicylat
12	0.50	0.68	0.74	0.80	0.84	0.95	25	34	0.73	
13							19	31	0.61	
14	0.66	0.81	0.82	0.81	0.79	1.03	26	34	0.77	No
15							27	31	0.87	Salicylat
16	0.55	0.78	0.71	0.80	0.81	0.99	9	22	0.39	

50 mg. ascorbic acid orally each day.

TABLE 19.

Effect of cortisone. Non-rheumatoid subject No. 2.

	PLASMA			ERYTHROCYTES			URINE			
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	AA + DHA mg./24 hrs.	Ratio $\frac{AA}{AA + DHA}$	Period
1	0.50	0.70	0.71	0.70	0.73	0.96	21	31	0.68	Low
2										Ascorbic
3							15	23	0.65	Acid
4	0.43	0.55	0.79	0.76	0.80	0.95	29	34	0.85	
5							29	36	0.81	Low
6	0.50	0.60	0.83	0.70	0.68	1.03	26	33	0.79	Ascorbic
7							18	23	0.75	Acid +
8	0.60	0.73	0.82	0.93	0.98	0.95	23	-	-	Cortisone
9							33	42	0.79	
10							35	43	0.81	Low Ascorbic
11	0.82	1.02	0.81	0.92	0.93	0.99	25	32	0.79	Acid + reducing
12							25	41	0.61	doses of
13	0.57	0.75	0.76	0.78	0.80	0.98	29	41	0.71	Cortisone.
14							26	44	0.59	Low
15							18	28	0.64	Ascorbic
16	0.52	0.73	0.71	-	0.68	-	-	23	-	Acid

50 mg. ascorbic acid orally each day.

TABLE 20.

Effect of salicylate. Rheumatoid arthritis subject No. 1.

	PLASMA			ERYTHROCYTES			URINE			
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	AA + DHA mg./24 hrs.	Ratio $\frac{AA}{AA + DHA}$	Period
1	0.12	0.32	0.38	0.36	0.42	0.86	16	19	0.84	
2							3	15	0.20	Preliminary
3	0.21	0.30	0.70	0.60	0.67	0.90	2	25	0.08	
4							17	28	0.61	
5	0.17	0.33	0.52	0.45	0.49	0.92	15	18	0.83	
6							30	36	0.84	Salicylate
7	0.16	0.28	0.57	0.52	0.58	0.90	18	31	0.58	
8							12	22	0.55	
9							23	27	0.85	Reducing
10	0.37	0.40	0.93	0.75	0.77	0.97	20	28	0.71	doses of
11							17	22	0.77	Salicylate
12	0.20	0.24	0.83	0.66	0.71	0.93	16	20	0.80	
13							19	30	0.63	
14	0.36	0.40	0.90	0.73	0.76	0.96	18	24	0.75	No
15							23	36	0.64	Salicylate
16	0.19	0.28	0.69	0.35	0.40	0.88	12	21	0.57	

50 mg. ascorbic acid orally each day.

TABLE 21.

Effect of cortisone. Rheumatoid arthritis subject No. 2.

	PLASMA			ERYTHROCYTES			URINE			
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	AA + DHA mg./24 hrs.	Ratio $\frac{AA}{AA + DHA}$	Period
1	0.19	0.38	0.50	0.84	0.90	0.93	20	28	0.71	Low
2										Ascorbic
3							11	25	0.44	Acid
4	0.28	0.33	0.85	0.44	0.43	1.02	16	24	0.67	
5							22	29	0.76	Low
6	0.32	0.32	1.00	0.56	0.55	1.04	11	14	0.79	Ascorbic
7							21	43	0.49	Acid +
8	0.22	0.50	0.44	0.53	0.57	0.93	26	28	0.93	Cortisone
9										
10							38	42	0.76	Low Ascorbic
11	0.37	0.47	0.79	0.58	0.61	0.95	27	34	0.79	Acid + Reducing
12							24	33	0.73	doses of
13	0.30	0.42	0.72	0.42	0.47	0.89	21	26	0.81	Cortisone.
14							22	34	0.65	Low
15	0.23	0.33	0.70	0.49	0.50	0.98	11	20	0.60	Ascorbic
16										Acid.
17							29	38	0.77	
18	0.21	0.43	0.49	0.46	0.50	0.92				

50 mg. ascorbic acid orally each day.

TABLE 22.

Effect of salicylate. Non-rheumatoid subject No. 3.

	PLASMA			ERYTHROCYTES			URINE			
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	AA + DHA mg./24 hrs.	Ratio $\frac{AA}{AA + DHA}$	Period
1	0.98	1.08	0.93	1.10	1.10	1.00	22	34	0.65	50 mg./day
2										Ascorbic
3							20	25	0.80	
4	0.78	1.05	0.74	1.07	1.10	0.97	33	49	0.85	Acid
5							230	307	0.75	500 mg./da
6	0.91	1.05	0.86	1.08	1.13	0.93	203	267	0.76	Ascorbic
7							278	347	0.83	
8	1.40	1.43	0.98	1.45	1.41	1.03	397	-	-	Acid
9							437	497	0.88	
10							506	551	0.92	500 mg./da
11	1.28	1.38	0.93	1.32	1.32	1.00	467	487	0.96	Ascorbic
12							429	435	0.96	
13	1 .07	1.38	0.78	-	-	-	375	416	0.90	Acid +
14							383	398	0.97	Salicylate
15	1.20	1.35	0.89	1.15	1.23	0.95	374	386	0.97	500 mg./da
16							277	308	0.90	Ascorbic
17							-	-	-	Acid
18	1.14	1.33	0.86	1.28	1.32	0.97	135	151	0.89	50 mg./day
19							74	83	0.89	Ascorbic
20	1.03	1.08	0.95	-	1.26	-	45	55	0.82	Acid

TABLE 23.

Effect of cortisone. Non-rheumatoid subject No. 4.

	PLASMA			ERYTHROCYTES			URINE	
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	Period
1	1.03	1.07	0.96	0.99	1.01	0.98	21	50 mg./day
2							19	
3	0.89	1.00	0.89	0.97	1.00	0.97	23	Ascorbic Acid
4							66	500 mg./day
5	1.89	1.90	0.99	1.47	1.47	1.00	222	Ascorbic Acid
6							437	
7	1.95	2.00	0.98	2.10	2.18	0.96	488	
8							418	
9							528	
10	2.12	2.13	1.00	1.91	1.88	1.02	384	500 mg./day
11							403	Ascorbic Acid
12	1.99	2.01	0.99	2.02	2.07	0.97	446	+ Cortisone.
13							453	
14							508	500 mg./day
15	1.91	1.85	1.03	2.14	2.10	1.02	432	Ascorbic Acid +
16							444	reducing doses of
17	1.91	1.89	1.01	1.72	1.72	1.00	303	Cortisone.
18							352	
19	2.07	2.08	0.99	1.92	1.91	1.01	367	500 mg./day
20							324	
21	2.02	2.03	1.00	1.97	1.96	1.00	448	Ascorbic Acid
22							332	
23							-	
24	1.89	1.90	0.99	1.60	1.68	0.95	423	
25							130	50 mg./day
26							24	
32	1.12	1.20	0.93	1.34	1.34	1.00	25	Ascorbic Acid.

TABLE 24.

Effect of salicylate. Rheumatoid arthritis subject No. 3.

	PLASMA			ERYTHROCYTES			URINE	
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	Period
1	0.43	0.58	0.74	0.98	1.04	0.94	21	50 mg./day Ascorbic Acid
2							21	
3	0.31	0.64	0.49	0.90	0.93	0.97	24	
4							21	
5	0.22	0.48	0.47	1.18	1.24	0.95	28	500 mg./day Ascorbic Acid
6							31	
7	0.56	0.74	0.76	1.11	1.19	0.93	25	
8							19	
9							283	
10	1.62	1.68	0.96	1.34	1.42	0.95	228	500 mg./day Ascorbic Acid + Salicylate.
11							182	
12	1.50	1.47	1.02	1.93	1.95	0.99	67	
13							178	
14							375	500 mg./day Ascorbic Acid + reducing doses of Salicylate.
15	1.65	1.56	1.06	2.03	1.96	1.03	410	
16							401	
17	1.43	1.43	1.00	1.89	1.94	0.97	303	
18							207	
19	1.26	1.28	0.99	1.71	1.71	1.00	181	
20							258	500 mg./day Ascorbic Acid
21	0.81	1.01	0.80	1.26	1.31	0.96	280	
22							236	
23							257	
24	0.95	1.27	0.75	1.46	1.49	0.98	298	
25							-	
26							21	50 mg./day Ascorbic Acid
32	0.50	0.66	0.76	1.13	1.17	0.97	19	

TABLE 25.

Effect of cortisone. Rheumatoid arthritis subject No. 4.

	PLASMA			ERYTHROCYTES			URINE	
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	AA mg./24 hrs.	Period
1	0.32	0.58	0.54	1.22	1.42	0.86	22	50 mg./day Ascorbic Acid
2							26	
3	0.42	0.82	0.52	-	1.31	-	21	
4							35	
5	0.43	0.58	0.74	-	1.04	-	34	500 mg./day Ascorbic Acid.
6							33	
7	0.65	0.76	0.86	1.00	1.14	0.88	40	
8							41	
9							315	
10	1.32	1.32	1.00	1.24	1.20	1.03	280	500 mg./day Ascorbic Acid
11							346	
12	1.12	1.25	0.90	1.20	1.49	0.81	346	+ Cortisone.
13							215	
14							349	500 mg./day
15	1.23	1.30	0.95	1.30	1.38	0.94	273	Ascorbic Acid +
16							265	reducing doses of
17	1.30	1.27	1.02	1.47	1.55	0.97	299	Cortisone.
18							289	
19	1.38	1.46	0.95	1.45	1.62	0.90	335	500 mg./day
20							288	Ascorbic Acid.
21	1.14	1.33	0.86	1.22	1.33	0.91	327	
28	0.53	0.70	0.76				-	50 mg./day Ascorbic Acid.

TABLE 26.

Effect of cortisone. Rheumatoid arthritis subject No. 5.

	PLASMA			ERYTHROCYTES	URINE			
Day	AA mg./100 ml.	TAA mg./100 ml.	Ratio $\frac{AA}{TAA}$	TAA mg./100 ml.	AA mg./24hrs.	AA + DHA mg./24 hrs.	Ratio $\frac{AA}{AA + DHA}$	Period
1					16	17	0.94	50 mg./day
2	0.27	0.52	0.52	0.87	14	18	0.78	Ascorbic Acid.
3					16	20	0.80	
4	0.24	0.30	0.80	0.22	21	28	0.75	
5					22	28	0.79	
6	0.13	0.25	0.52	0.58	33	46	0.72	500 mg./day
7					-	-		Ascorbic Acid
8					31	39	0.80	
9	0.55	0.63	0.87	1.17	33	43	0.77	
10					124	152	0.82	
11	0.95	1.13	0.84	0.93	230	332	0.69	
12					482	595	0.81	500 mg./day
13	1.23	1.53	0.80	1.10	439	466	0.94	Ascorbic Acid
14					-	-		+ Cortisone.
15					194	219	0.89	
16	1.32	-	-	1.05	273	338	0.81	
17					406	462	0.88	500 mg./day
18	1.10	1.30	0.85	1.28	275	332	0.82	Ascorbic Acid +
19					226	256	0.88	reducing doses of
20	1.25	1.38	0.91	1.05	506	549	0.92	Cortisone.
21					-	-	-	
22					394	521	0.72	500 mg./day
23	1.08	1.26	0.94	1.58	456	585	0.78	Ascorbic Acid.
24					252	305	0.83	
25	1.18	1.32	0.89	1.07	267	371	0.72	
26					173	200	0.87	
27					52	62	0.84	50 mg./day
28					-	-	-	Ascorbic Acid
29					41	48	0.85	
30	0.62	0.72	0.86	1.26	27	43	0.63	
31					28	38	0.74	
32	0.51	0.62	0.82	1.12	25	36	0.69	

TABLE 27.

Effect of salicylate. Rheumatoid arthritis

subject No. 6.

Day	Plasma TAA mg/100 ml.	Erythrocyte TAA mg./100 ml.	Urine TAA mg./24 hours	Period
1	0.23	0.57	7	Ordinary
3			4	hospital
5			8	diet.
7			5	
8	0.37	0.80		
9			3	
11			9	
13			4	
15	0.28	0.91	4	Ordinary
17			86	hospital diet
19			258	+ 500 mg./ day
21			124	ascorbic acid.
22	1.20	1.55		
23			256	
25			378	
27			332	
29	1.41	1.10	465	Ordinary
31			335	hospital diet,
33			162	500 mg./day
35			112	ascorbic acid +
36	1.04	1.10		salicylates.
37			265	
39			315	
41			350	
43	1.20	0.87	275	Ordinary
45			386	hospital diet
47			334	+ 500 mg./day
49			151	ascorbic acid
50	0.79	0.76		Ordinary
51			72	hospital diet
53			14	
55			10	
57	0.40	0.62		

TABLE 28.

Effect of salicylate. Rheumatoid arthritis subject No. 7.

Day	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Urine TAA mg./24 hours.	Period.
1	0.17	0.54	6	Ordinary Hospital diet
3			1	
5			4	
7			5	
8	Nil	0.05		
9			4	
11			6	
13			10	
15	0.10	0.25	3	Ordinary hospital diet + 500 mg./day ascorbic acid
17			12	
19			18	
22	1.45	1.49		
23			306	
25			300	
27			320	
29	1.30	1.54	409	Ordinary hospital diet + 500 mg./day ascorbic acid + salicylate.
31			314	
33			291	
35			328	
36	1.12	1.29		
37			274	
39			376	
41			261	
43	0.93	1.15	291	
45			285	
47			180	
49			131	
50	0.80	1.10		Ordinary hospital diet + salicylate.
51			43	
53			15	
55			16	
57	1.17	0.70		

Note:- By mistake, the patient was kept on salicylate, 80 grains per day, from day 29 to end of test period.

TABLE 29.

Effect of salicylate. Rheumatoid arthritis subject No. 8.

Day	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Urine TAA mg./24 hours	Period
1			8	Ordinary hospital diet
2	0.20	0.44	-	
3			8	
5			11	
7			4	
8	0.09	0.38		
9			1	
11			6	
13			6	
15	0.11	0.32	2	Ordinary hospitaldiet + 500 mg./day ascorbic acid.
17			5	
19			16	
21			274	
22	1.56	1.82		
23			292	
25			260	
27			300	
29	1.13	-	220	Ordinary hospital diet, 500 mg./day ascorbic acid + salicylate
31			330	
33			349	
35			275	
36	1.32	1.75		
37			150	
39			420	
41			382	
43	1.24	-	315	Ordinary hospital diet + 500 mg./day ascorbic acid.
45			205	
47			358	
49			288	
50	0.99	1.40		Ordinary hospital diet
51			27	
53			6	
55			3	
57	0.54	0.77		

TABLE 30.

Effect of cortisone. Rheumatoid arthritis subject No. 9.

Day	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Urine TAA mg./24 hours	Period
1	0.20	0.56	6	Ordinary
3			3	hospital
5			5	diet
7			2	
8	0.22	0.38		
9			5	
11			8	
13			4	
15	0.24	0.52	2	Ordinary
17			9	hospital diet
19			170	+ 500 mg./day
21			292	ascorbic acid.
22	0.90	1.53		
23			444	
25			428	
27			235	
29			715	Ordinary
31	1.03	1.34	525	hospital diet,
33			299	500 mg./day
35			489	ascorbic acid
36	1.18	1.62		+ cortisone.
37			520	
39			308	Ordinary hospital diet, 500 mg./day ascorbic acid + reducing doses of cortisone.
41			327	
43			575	Ordinary
45	0.73	0.86	398	hospital diet
47			408	+ 500 mg./day
49			492	ascorbic acid.
50	1.17	1.60		Ordinary
51			43	hospital
53			2	diet.
55			1	
57	0.39	0.77		

TABLE 31.

Effect of cortisone. Rheumatoid arthritis subject No. 10.

Day	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Urine TAA mg./24 hours	Period
1	0.08	0.23	4	Ordinary hospital diet.
3			3	
5			3	
7			3	
8	0.22	0.56		
9			5	
11			1	
13			4	
15	0.14	0.37	2	Ordinary hospital diet + 500 mg./day ascorbic acid.
17			9	
19			31	
21			141	
22	1.20	1.64		
23			264	
25			274	
27			504	
29	1.23	0.98	393	Patient on anti-coagulant treatment for phlebitis - high ascorbic acid dosage continued.
31			288	
33			215	
35			90	
36	1.42	1.42		Hospital diet, 500 mg./day ascorbic acid + cortisone.
37			239	
39			495	
41			300	
43	1.42	2.40	334	
45			780	
47			335	
49			620	
50	1.44	1.67		Ordinary hospital diet + 500 mg./day ascorbic acid.
51			244	
53			165	
55			300	
57	1.16	-	48	Ordinary hospital diet
59			11	
61			8	
64	0.85	1.45		

TABLE 32.

Effect of cortisone. Rheumatoid arthritis subject No. 11.

Day	Plasma TAA mg./100 ml.	Erythrocyte TAA mg./100 ml.	Urine TAA mg./24 hours	Period
1	0.03	0.46	6	Ordinary hospital diet
3			3	
5			4	
7			1	
8	0.10	0.08		
9			4	
11			11	
13			3	
15	Nil	0.10	13	Ordinary hospital diet + 500 mg./day ascorbic acid.
17			10	
19			9	
21			45	
22	1.12	1.63		
23			294	
25			324	
27			278	
29	0.97	1.84	332	Ordinary hospital diet, 500 mg./day ascorbic acid + cortisone.
31			268	
33			388	
35			240	
36	1.08	1.08		
37			515	
39			203	
41			425	
43	0.67	0.92	198	Ordinary hospital diet, + 500 mg./day ascorbic acid.
45			308	
47			180	
49			87	
50	0.80	1.18		Ordinary hospital diet
51			56	
53			20	
55			9	
57	0.40	0.45		